Function and organization of courtship behaviour in *Drosophila melanogaster*

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Abstract

The courtship behaviour of both male and female Drosophila melanogaster changes with time. The durations and probabilities of occurrence of the various acts are affected. Some of the behaviour patterns show cycles in their bout durations. The patterns of change of the male and female behaviour show little evidence of interaction betwen the sexes. All the measured aspects of courtship song change significantly with time.

Detailed analysis of the behavioural sequences during courtship indicates that some female acts have significant effects on the male's behaviour and vice versa. Transitions in the behaviour of one sex cause a general decrease in the duration of the ongoing behaviour and a general decline in the probability of starting a new bout of behaviour in the other sex. Principal component analysis and canonical correlation analysis supported the conclusion that although there is significant male-female interaction these interactions do not have large effects on the behaviour of the flies.

A unitary motivational model for the courtship behaviour of the male was not supported. At least three underlying variables are required to explain the variation in the male's behaviour.

The courtship behaviour of male D. melanogaster is highly determined. From a knowledge of the male's and female's behaviour from the start of the observation period it is possible to significantly predict the future behaviour of the male and the remaining time till copulation.

Declaration

The data in Chapter 5 were collected in collaboration with F. von Schilcher. However, their analysis and interpretation were entirely my own. The remainder of the thesis is my work. I declare that this thesis has been composed by myself.

Acknowledgments

I thank Professor A. Manning, my supervisor, for his patience and, Dr. A. Ewing and F. von Schilcher for many discussions. I appreciate the comments of Dr. John Deag and Aubrey Manning. Mrs. C. Assumpcao typed the references and Robert Duncan drew five of the figures; I am grateful to them both. The list of friends who have encouraged me is too long to quote. I hope they all understand that I truly appreciate their concern.

To Barbara, in memoriam

"I have no faith in anything short of actual measurement"

Darwin

"it is not informative to study variations of behaviour unless we know beforehand the norm from which the variants depart"

Medawar

"If 'everybody knows' such-and-such, then it ain't so, by at least ten thousand to one."

Heinlein

"General impressions are never to be trusted. Unfortunately when they are of long standing they become fixed rules of life and assume a prescriptive right not to be questioned. Consequently those who are not accustomed to original inquiry entertain a hatred and a horror of statistics. They cannot endure the idea of submitting their sacred impressions to cold-blooded verification. But it is the triumph of scientific men to rise superior to such superstitions, to desire tests by which the value of beliefs may be ascertained, and to feel sufficiently masters of themselves to discard contemptuously whatever may be found untrue."

Galton

"The exact standardisation of experimental conditions, which is often thoughtlessly advocated as a panacea, always carries with it the real

disadvantage that a highly standardised experiment supplies direct information only in respect of the narrow range of conditions achieved by standardisation. Standardisation, therefore, weakens rather than strengthens our ground for inferring a like result, when, as is invariably the case in practice, these conditions are somewhat varied."

Fisher

"additive components usually account for a large part of the variation even in complex interactions"

Rao et al.

"psychohistory . . . that branch of mathematics which deals with the reactions of human conglomerates to fixed social and economic stimuli . . . can predict . . . history . . . five hundred years into the future . . . is a statistical science and cannot predict the future of a single man with any accuracy"

Asimov

"Model builders are content to rest their case on argument and persuasion, and models become dogmas and not stepping-stones to wider experimentation."

Green

"No measurement of single characters or environmental conditions in

can be cosidered in isolation without losing much of their significance."

Wright

"Our understanding of the behavioral aspects of mating is so unsatisfactory, though, that we are unable to advance explanations concerning the distribution of mating frequencies."

Wallace

"The phenotypes which develop in response to environmental influences which recur regularly in the normal habitats of species are usually adaptive and conducive to survival. The reactions to environmental stimuli which the species encounters rarely or never in its normal habitats are, on the contrary, seldom adaptive."

Dobzhansky

"a good deal of scepticism in a scientific man is advisable"

Darwin

Chapter 1. Introduction

The goal of science is to completely understand natural phenomena; for ethology this means understanding the behaviour of animals under relatively natural conditions. One way of determining the extent of our knowledge of any given behavioural system is by evaluating the magnitude of the discrepancies between predictions derived from hypotheses or models (e.g. Dawkins and Dawkins, 1974) and the actual responses of the system in various situations. Thus, "When enough characters have been considered to reduce the uncertainty about all stages of a fight to near zero, the analysis is complete - the descriptive model of factors controlling a fight is complete" (Hazlett and Estabrook, 1974a). However, we must bear in mind that "If prediction of behaviour, given the antecedent conditions, was the sole aim, there might be no need to reduce to a physiological level: reference to underlying mechanisms could be unnecessary. But even if complete prediction of behaviour were possible, we should still have advanced only one stage towards its full understanding" (Hinde, 1970). Ethology has not yet advanced to that stage, although progress is being made (e.g. Hazlett and Estabrook, 1974b; Fentress, 1972; 1973).

Mathematical approaches to ethology

Kloot and Morse (1975) suggested that one of the problems in the study of behaviour until recently has been the relative lack of adequate methods of data analysis. This is certainly no longer true. Mathematical methods have been diffusing into ethology from other areas of science at an ever increasing rate beginning with the introduction of information theory from engineering by Haldane and Spurway in 1954 for the study of the process of communication in

honeybee dances.

This analytical method has achieved particular prominence, and papers dealing with behavioural interactions or sequences use information analysis routinely (Pruscha and Maurus, 1976; Northup, 1977). But see Catchpole (1976) for one example of a sequential analysis which did not use information theory for a good reason - a song matrix with over 2000 cells. It will be interesting to see whether the recent generalisation of information analysis to include non-behavioural characters will prove as popular (Rubinstein and Hazlett, 1974).

Ethology has borrowed some of these statistical methods from psychology thus further reducing the differences between the two fields. For example, factor analysis has been used to shrink a large number of variables into a smaller number, usually three or less, of composite variables (Burton, 1970; Baerends and Cingel, 1962). Multidimensional scaling, a non-metric method of grouping behaviour patterns by their similiarity has also been borrowed from psychology (Guttmann, et al., 1969; Golani, 1973; Miller, 1975). A similar metric technique for grouping behaviours or animals by means of their similarity, cluster analysis, has been introduced from numerical taxonomy (Dawkins, 1976; Cheke, 1977; Morgan, et al., 1976; Maurus and Pruscha, 1973).

Principal components, the biological analogue of factor analysis, has also been applied to ethological problems (Dudzinski and Norris, 1970). This technique has fewer restrictive assumptions than factor analysis (Marriott, 1974), and it has been suggested that principal components be used in favour of factor analysis (Blackith and Reyment, 1971). Discriminant functions, a method used to combine many variables into a composite variable that best distinguishes

between given groups, (introduced from numerical taxonomy) have been used to examine behavioural differences between classes of animals (Frey and Miller, 1972; Brothers and Michener, 1974; Dow, et al., 1976), and Heckenlively (1970) used multiple regression in a similar way to differentiate between winners and losers of agonistic interactions in crayfish.

Stochastic analysis, using statistical methods based on the analysis of series of events has also been applied to animal behaviour (Delius, 1969; Kloot and Morse, 1975; Heiligenberg, and coworkers, 1966, 1973, 1976) as have methods for cyclic behaviour (Campbell and Shipp, 1974; Slater, 1975). Behavioural models based on grammatical concepts are also gaining favour (Vowles, 1970; Dawkins, 1976). McFarland and his co-workers (1974) have applied the methods of systems analysis to maintenance activities, and these methods are now being applied to more traditional ethological subjects, e.g. sexual behaviour of newts (Halliday, 1976).

As is to be expected with methods that have been borrowed from other fields most of these statistical methods should be used with caution when applied to ethological data. Critical discussions of the assumptions and shortcomings associated with the statistical analysis of behavioural data can be found in Slater (1973), Balthazart (1972, 1973), Chatfield (1973), Chatfield and Lemon (1970), and Cane (1956, 1959, 1961). However, judicious use of a wide variety of analytical techniques should maximally exploit the information present in behavioural data and provide the most complete description possible. This is particularly true in a discipline in which large quantities of data are easily generated with little possibility of being able to randomise all but one aspect of the experimental situation, especially the behavioural aspects.

Many of the published analyses suffer from a number of shortcomings; these are not unexpected in a young field such as mathematical or quantitative ethology. Thus, some analytical techniques have been applied to data sets which were collected before a decision had been made as to the method of analysis and therefore, were not collected with optimal designs (Delius, 1969). Other investigations have merely restated the earlier qualitative conclusions of ethologists in statistical terms, hopefully, more objectively (e.g. Balthazart, 1973).

Many investigations have stopped at the descriptive stage and have not attempted to use their findings to falsify existing models or hypotheses (Popper, 1972) However, many of these investigations are the first on particular topics or particular species and there may be no appropriate a priori hypotheses or models with which to order the results of the quantitative analyses (and ad hoc hypotheses are frowned upon). Often only one aspect of the behaviour has been examined and in only one particular way so that not enough information is available to produce a coherent hypothesis.

There have been a number of papers whose purpose has been more to introduce ethologists to a new technique than to use that technique to provide new information about the organisation of behaviour.

It should be stressed that quantitative ethologists must be adept at avoiding statistical pitfalls. This especially applies to lack of independence in statistical tests, thus biasing their probabilities (e.g. Siegel, 1956, p. 159) and the use of ratios.

Ratios (percentages, proportions, etc.) should never be used (statistically analysed) unless the experimenter is absolutely certain (mathematically) that spurious correlations and biases are not

being introduced into the data by so doing (Atchley, et al., 1976). In all cases alternate statistical methods exist which can answer the same questions without bias, e.g. analysis of covariance, principal components, multivariate analysis of variance, etc.

Ethology of Drosophila

The courtship behaviour of Drosophila is well suited as subject material for quantitative analyses of the organisation of behaviour. There are only four basic male courtship behaviour patterns (orientation, vibration, licking, attempted copulation) and seven female patterns (standing, flicking, kicking, extruding, elevating, preening, fending). The courtship requirements of Drosophila can be met in the laboratory, large data sets are collected relatively easily and there are a priori hypotheses available in the literature with which to structure the results of the statistical analyses.

There have been two quantitative analyses of courtship behaviour in the genus Drosophila. One study (Bastock, 1956) was primarily concerned with the behaviour of male D. melanogaster. Bastock and Manning (1955) put forward a three threshold model to explain the patterning of elements of male behaviour. They postulated an underlying "motivational tendency" (M) and three fixed thresholds. When a male's "motivational" value was above the lowest threshold orientation was performed; above the second threshold vibration appeared, and thus was superimposed upon orientation, and above the highest threshold licking appeared and was superimposed upon vibration.

A later study by Brown (1964) examined the courtship behaviour of D. pseudoobscura males in detail. Brown found that the three threshold model (Bastock and Manning, 1955) was not applicable to D. pseudoobscura. Although the courtship of D. melanogaster could be

explained with three thresholds and one underlying "motivational" tendency, that of D. pseudoobscura required interaction between two tendencies, one for copulation and the other for avoidance.

Unfortunately, the three threshold model has been criticised on statistical grounds (Cane, 1959, 1961). Cane re-examined the data of Bastock and Manning (1955) and concluded that the courtship behaviour of D. melanogaster could be adequately explained by a random effects model without postulating thresholds or "motivational" tendencies.

Work on the courtship behaviour of yellow mutant males has produced anomalous results in terms of the threshold model. Yellow males of D. melanogaster have a lower tendency to court females than wild-type males, and the model predicts that this should result in their showing more orientation, less vibration and less licking than wild-type males. Although the predictions are borne out for orientation and vibration they are contradicted for licking; yellow males lick as often as wild-type males (Bastock, 1956; Dow, 1976).

Recent research (Connolly and Cook, 1973) has suggested that the female does not play an important role in determining the behaviour shown by courting D. melanogaster males. Connolly and Cook (1973) found that the major effect of the female's behaviour during courtship was to prevent physical contact (e.g. licking, attempted copulation) by the male. However, Brown (1964) found that in D. pseudoobscura extrusion by the female inhibited male courtship. Bastock and Manning (1955) also concluded that extrusion by female D. melanogaster inhibited male courtship behaviour. But Connolly and Cook (1973) noted that Bastock and Manning's data supported their own observations that males often attempted to copulate while a female was extruding and thus extrusion was not immediately inhibiting.

Cook (1973) found that female receptivity was strongly related

to locomotor activity in D. melanogaster, and Schilcher (1976a) showed that auditory stimulation with courtship song caused 3 day old mature D. melanogaster females to slow down their rate of running and that this effect was not present in one day old unreceptive females. In a factorial study of mating speed in five strains of D. melanogaster Parsons (1965) found that mating speed was determined solely by the male. However, Cook (1973) using the same species found that time to copulation was at least partially female-determined.

It has been postulated that female receptivity is determined by two independent systems: a switch mechanism and a summation mechanism (Manning, 1967). Females which are physiologically sexually mature due to their hormone levels are "switched-on" but must summate the various stimuli produced by courting males for some period of time (courtship time, mating speed) before they will accept a male and allow copulation to occur (Manning, 1967). The summation hypothesis is presumably analogous to the concept of heterogeneous summation (Tinbergen, 1951) but occurs over time.

The following quote indicates some of the evidence upon which the summation hypothesis was based. "As several minutes of vibration are usually required before the female will copulate one assumes that vibration does not merely have a 'trigger' function but acts additively in facilitating copulation" (Ewing, 1964). It has also been found that time to copulation is linearly related to wing area in D. melanogaster (Ewing, 1964) and that even males with no wings will mate if allowed enough time and no competition from winged males (Averhoff and Richardson, 1974).

One alternative to a summation hypothesis is a trigger hypothesis that assumes that female Drosophila have specific stimulatory requirements (such as hearing vibration bouts of particular intensi-

ties and of specific minimum durations, etc.) and that they allow males to mate only after all their requirements have been met. Only if all repetitions of the male behavioural elements during courtship were identical in their stimulatory value to the female would the evidence available now distinguish between these two hypotheses.

It is now known that bouts of the pulse song component of courtship song of D. melanogaster are not identical in stimulatory value. The inter-pulse interval (ipi) of this song is strongly related to mating speed in D. melanogaster (Schilcher, 1976b; Bennet-Clark and Ewing, 1969) and has been shown to increase with time during courtship (Wilson, et al., 1976) and to be significantly different between males (Schilcher, 1975).

Schilcher (1976b) has determined that pulse song cannot be summated by the female as its effects are not stored for any length of time. A second component of courtship song, sine song, is stored by the female and thus may, possibly, be summated. It is also likely that bouts of both songs differ in intensity throughout a courtship since, for example, males sing from different distances from the female at various times.

Furthermore it has recently been suggested that the summation mechanism and "switch-on" mechanism are related (Cook, 1973).

Aims

The research in this thesis has been undertaken with the following goals:

1) To describe the courtship behaviour of both male and female D. melanogaster as completely as possible under a particular set of laboratory conditions. Schilcher (1975) was concerned with the auditory aspects of courtship behaviour in the same species.

2) To use the descriptive results to test the predictions of the three-threshold model of courtship behaviour and to examine the summation hypothesis in wild-type courtships.

3) To determine the relationship between the various elements of courtship behaviour and the time to copulation.

Chapter 2. Materials and methods

Two strains of Drosophila melanogaster were used in these experiments. One strain, with attached X-chromosomes, was used only for the analyses of courtship song in Chapter 5, and is described there. The other, a wild-type strain, was collected as 60 females in Lawrence, Kansas in October, 1971 by Dr. P. W. Hedrick. This stock was used for the analyses in all Chapters other than 5. The Kansas population was cultured in a population cage until immediately prior to experimentation when egg samples were taken from the cage and cultured in half-pint milk bottles containing yeasted cornmeal-molasses-agar-Nipagin medium (UFAW Handbook, 1959). Cultures were kept at $25^{o} \pm 3$ C under a 12:12 light:dark regime. All flies were collected as virgins by ether anaesthetisation within 12 hr. of eclosion and maintained singly in glass vials (22 X 57 mm) containing yeasted medium for 3 to 7 days. Most tests were conducted on 4 day old pairs.

Courtships were observed by introducing pairs of flies into Perspex cells (13 mm internal diameter, 6 mm high) without etherisation. The sexes were kept separated by a movable partition for 10 - 15 min. to settle them. The partition was then removed and the entire courtship was videotaped with a Nivico 820R recorder and a Phillips TV camera. To insure that the entire courtship was observed, recording started before the partition was removed.

A recording session lasted until one videotape was full. A videotape could hold a maximum of 55 min. of courtship. Any pair which had not started to court within 5 min. from the start of recording or had not copulated within 5 min. of the start of courtship was discarded and the tape for that pair was erased. A preliminary analysis indicated that for the wild-type strainS Mancha Vinegar and Malawi, 93% of the pairs had copulated within this time

limit. This compares favourably to the 89% of pairs of flies of these and similar strains that will ever copulate when placed in glass vials with medium and left for 9 days (Dow, 1976) and therefore little if any bias will have been introduced by this procedure.

Observation cells were washed in warm water after each recording session.

Behaviour patterns were recorded using a Peebles-Edgecumbe event recorder on playback of the videotapes. On the first playback male courtship patterns were recorded and on the second playback female behaviours were recorded. The two playbacks were synchronised by the time at which the partition was removed from the cell.

The following behaviour patterns were recorded:

Male

Non-courtship (NON): running, preening, standing - male not engaged in any of the following behaviours or if so engaged not facing the female and not within approximately one body length of her.

Orientation (ORI): male facing the female and not vibrating, licking or attempting to copulate.

Vibration (VIB): male facing the female and one or both wings not resting on the dorsal surface of the abdomen. Schilcher (personal communication) found that during wing scissoring normal pulse song was produced. This may also occur during wing flicking. Vibration bouts may contain pulse song bouts, sine song bouts and intervals of silence. In many bouts of vibration the male begins by scissoring both wings with ever greater amplitudes and then switching to flicking one wing to ever greater amplitude until that wing is extended to a 90 degree angle with the body and vibrated vertically

more or less continuously for a period of time. However, such vibration may also occur during scissoring and flicking although more difficult to observe due to the horizontal motion of the wing(s).

Licking (LIC): male licks the female's genitalia with his proboscis. Intention licks which clearly failed to make contact with the female were not recorded. The male may follow the female with his proboscis partially extended to various degrees.

Attempted Copulation (ATT): the tip of the male's abdomen is curled under and touches the tip of the female's abdomen. Intention attempted copulations which did not clearly touch the female were not recorded.

Female

Standing (STA): the female stands totally still.

Running (RUN): the female is moving at any speed.

Preening (PRE): the female preens any part of her body.

Fending (FEN): the female gently touches the male with one of her legs.

Kicking (KIC): the female vigorously kicks the male. This is typically done with both hindlegs although occasionally only one hindleg may be used.

Flicking (FLI): the female quickly extends and retracts one or both wings to a variable extent usually less than 45 degrees. When both wings are extended they may be slightly tilted. This posture is occasionally held for several seconds.

Elevating (ELE): the female raises the tip of her abdomen away from the horizontal.

Extruding (EXT): the female extrudes her ovipositor toward the male.

A female may also curl her abdomen beneath her (Bastock and Manning, 1955) but this was rarely observed and therefore, not recorded. Spreading of the vaginal plates is also seen in D. melanogaster females. However, due to the difficulty in observing the slight changes in the appearance of the abdominal tip that is produced by spreading, this behaviour was not recorded. Careful observation of a few courtships suggests that all females spread their plates during courtships, that spreading may occur more than once during a courtship and that males may not use this behaviour as a signal to copulate.

The following data were obtained from the event records: the time from the removal of the partition to the start of courtship (courtship latency), the time from the start of courtship to copulation (courtship duration, mating time, mating speed), the time of the start and of the end of all behavioural acts.

A bout was defined as the time during which a behavioural element was apparently continuously displayed by the animal. Bout durations were measured for ORI, VIB, NON, ATT, STA, RUN, ELE, EXT, and PRE. An interval was defined as the time from the end of one act until the start of the next act of that behaviour pattern, regardless of whether acts of the other behaviour patterns intervened.

All measures were normalised by transformation before statistical analysis if they were not originally normally distributed. The transformations are statistically validated in Appendix 1. All results will be presented in the transformed scales only.

The data analyses involve both univariate and multivariate statistics. Since the latter have been infrequently used in ethological studies a short description of the methods follows.

Univariate statistics is concerned with the distribution of

single variables and multivariate statistics is concerned with the joint distribution of several variables. In controlled experiments one variable can be manipulated and by randomisation, combined with uncorrelated values of all other variables. However, in the observational studies that play a major role in ethology such control is not possible and thus variables are correlated. It then becomes difficult, if not impossible, to specify the effects of each variable with simple univariate analyses.

Multiple regression

This technique is considered a univariate method and relates a single dependent variable to a number of independent variables. It is a generalisation of simple regression techniques which involve only one dependent variable (Y) and one independent variable (X). Multiple regression can be considered an extension of the algebraic solution of n equations in n unknowns to the solution of n equations in m unkowns, n>m, using least squares to provide a unique solution (Kerlinger and Pedhazur, 1973). Beer (1963) used an analogous method, partial correlation, to determine the relationships between the behaviour patterns involved in incubation.

Canonical analysis

This includes both canonical correlation and canonical variates and is a generalisation of multiple regression to the problem of relating two sets of variables to each other. It can for example, be used to relate the set of variables, female Drosophila behaviours to the set of male behaviours. Canonical analysis attempts to explain the shared variance (covariance) between two sets of data by extracting an initial composite variate (the canonical variate) that

accounts for the largest proportion of the variance; if significant, further composite variates which are uncorrelated are extracted until no more significant variates can be extracted.

Factor analysis and principal components analysis

Both these methods are designed to reduce a set of multivariate data to a small number of underlying factors or components. Principal components are always independent. Factors are initially independent but it is usual to modify them by rotating their axes. After oblique rotations the resulting factors are correlated; they remain uncorrelated after orthogonal rotations. Factor analysis involves fitting a definite mathematical model to results and was originally developed by psychologists for use in intelligence research (Hope, 1968). Principal components are used by biologists and have been recommended by Blackith and Reyment (1971). Huntingford (1976) has given an excellent introduction to principal components analysis.

Chapter 3. Courtship model for Drosophila melanogaster males

In ethology models have often served as useful "hypotheses" from which predictions can be made, experiments designed and our knowledge of behaviour critically tested (Lorenz, 1950; Tinbergen, 1951). The courtship behaviour of Drosophila melanogaster males has been modelled by Bastock and Manning (1955; Bastock, 1956) who based their model on detailed analyses of wild-type and mutant yellow male courtships. Brown (1964) examined the courtship behaviour of male Drosophila pseudoobscura and found that it did not fit the model for D. melanogaster. He therefore, provided an alternative hypothesis for the organisation of courtship behaviour in male D. pseudoobscura. Eastwood (1974) has recently reexamined the D. melanogaster model and has slightly modified it to include attempted copulation.

Figure 1 (from Bastock, 1956) represents the initial model. It is based on three assumptions, "(1) that the motivation fluctuates regularly, (2) that the elements occur at different thresholds of this motivation, and (3) that during courtship the motivation remains for most of the time within the limits set by these thresholds" Bastock (1956).

The model assumes an underlying variable M, called sexual motivation, that in some manner determines the behaviour of the male. The end result of the behavioural mechanism linking M to courtship behaviour is that when M fluctuates above a behavioural threshold that behaviour is shown by the male. This model explains well the superposition of the three behaviours, orientation, vibration and licking; vibration occurs when the male is orientated and licking occurs when the male is vibrating. However, there are rare occasions when a male may vibrate and lick while not orientated to a female, although he may nevertheless be orientated to something, for example,

Figure 1. Courtship model for D. melanogaster males (from Bastock (1956)).

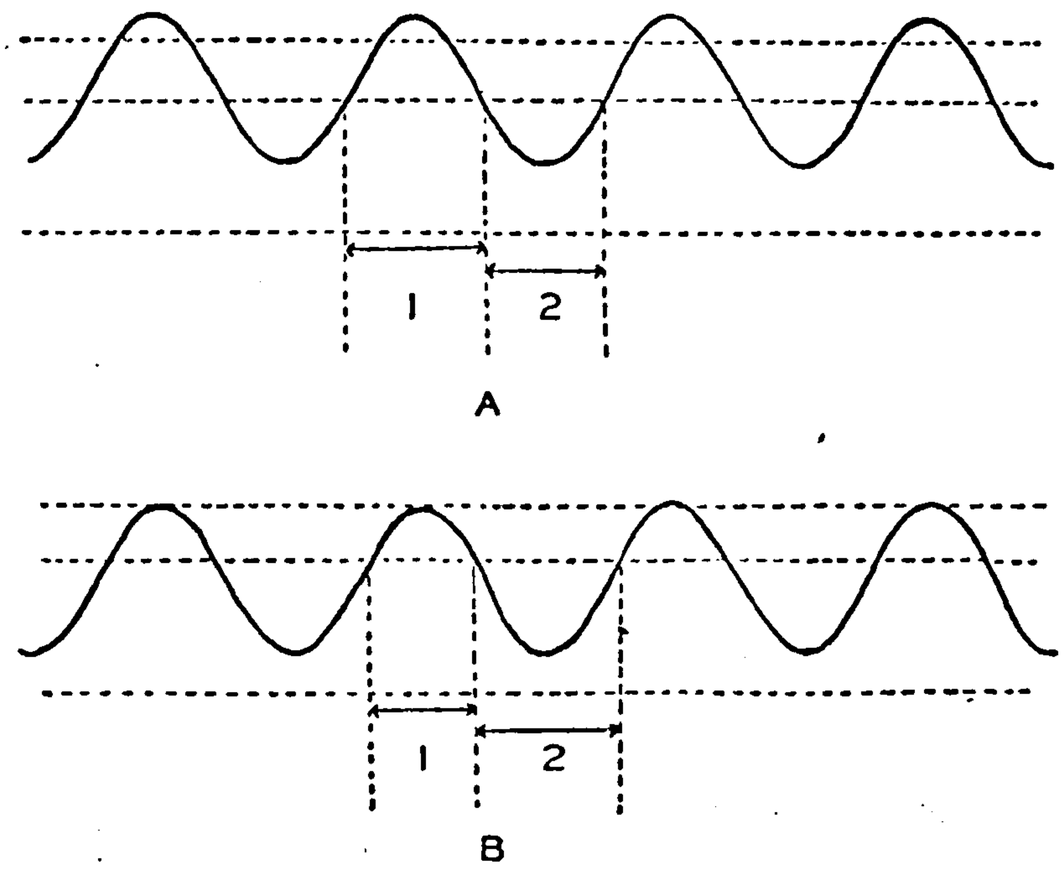


Diagram of the suggested way in which a change in average sexual motivation affects the courtship pattern. Motivation is assumed to fluctuate regularly in relation to the three thresholds at which the elements appear. A. High average motivation produces courtship typical of wild type males. B. Low average motivation produces courtship pattern typical of yellow males. 1. Bout length of vibration and licking. 2. Bout length of orientation.

female odour or a visual stimulus such as a lump of yeast.

The threshold model makes three predictions about the details of wild-type male courtships

(1) vibration bout length and orientation bout length are negatively correlated

(2) vibration boutlength and licking rate are positively correlated

(3) vibration boutlength is positively correlated with total response time - the amount of time a male courts a female in a fixed amount of time. In the original case the fixed amount of time was 5 hours. These predictions were verified (Bastock, 1956; Bastock and Manning, 1955).

Eastwood (1974) has argued that since boutlengths of vibration and orientation are variable, and although there is a strongly favoured sequence of behaviour, i.e. ORI -> VIB -> LIC -> ATT, that this sequence is far from invariable and therefore, the assumption that motivation M fluctuates regularly should be discarded and motivation allowed to fluctuate irregularly. Thus, motivation may be represented as a random waveform (Figure 2).

From this modified model a generalised model can be derived with assumptions as follows,

1) Sexual motivation fluctuates. These fluctuations need not be regular nor continuous. The overall time spent at each given value of M may be summed to produce a probability distribution of M for each male.

2) These probability distributions are the same shape for all males.

3) The probability distributions are unimodal. The distribution diagrammed in Figure 3 is normal and thus symmetrical about its mean.

Figure 2. A random waveform.

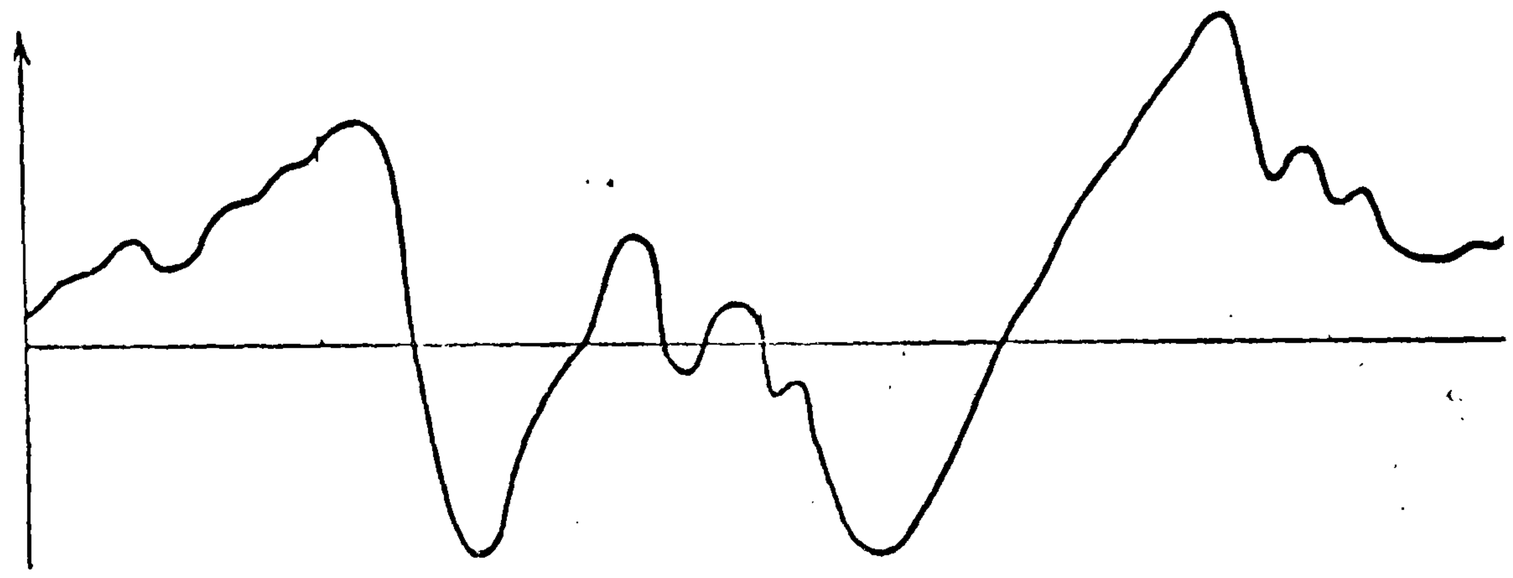
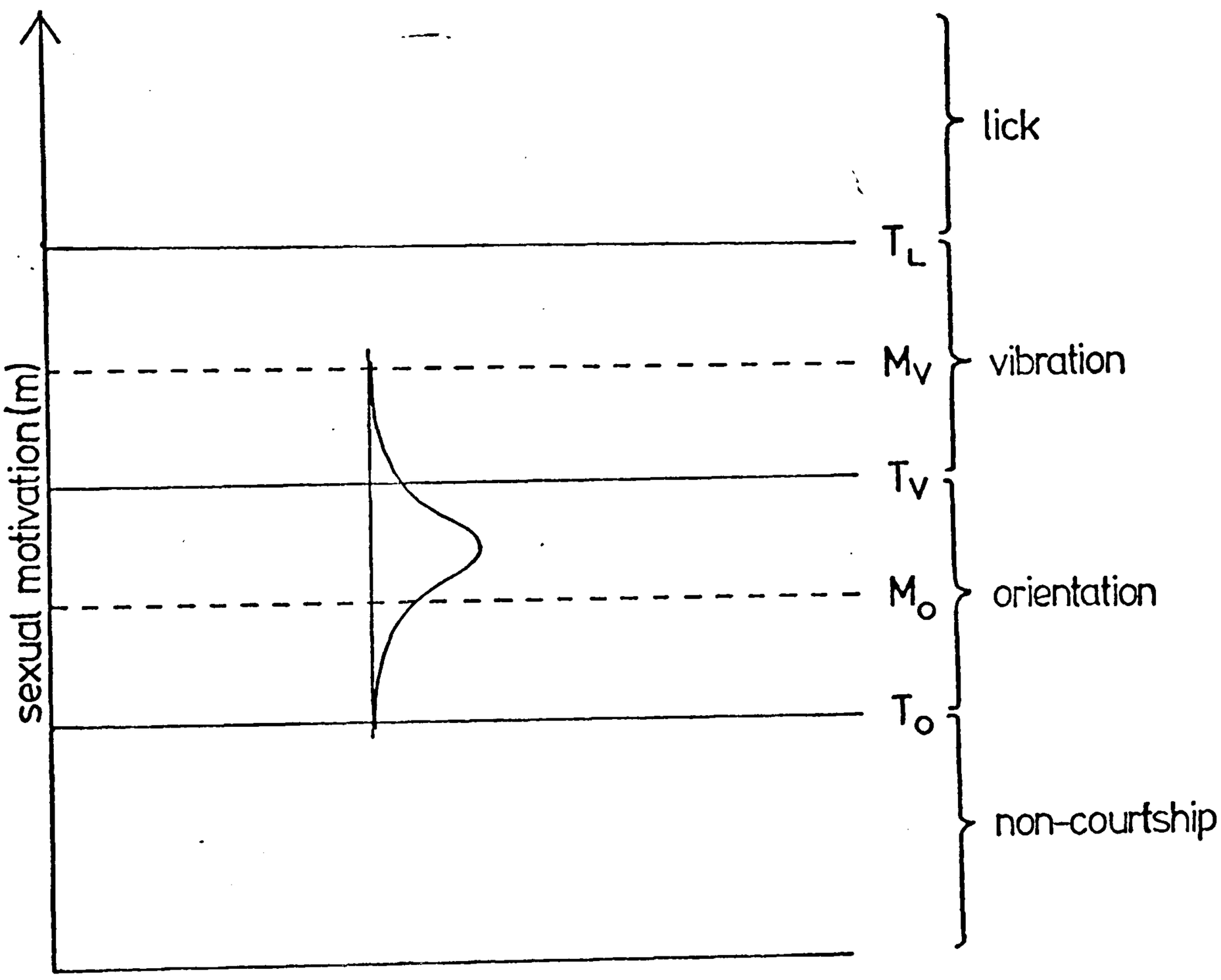


Figure 3. Modified model for the courtship behaviour of D. melanogaster males. T represents a threshold; M represents the midpoint of an interval; the normal curve represents the probability distribution of sexual motivation for a male.



However, the predictions are not altered qualitatively by assuming other unimodal distributions including asymmetrical ones. The model is robust to this assumption.

4) The thresholds are fixed at the same values of M for all virgin males.

5) Every male can be characterised by a mean M and a variance of M.

6) The mean level of M may change during courtship; it need not be stationary.

This generalised model contains as sub-models both the original model (Bastock, 1956) and the modified model of Eastwood (1974). It follows Eastwood in assuming that successful licks (that touch the female) trigger complete attempted copulations. Predictions about the courtship behaviour of D. melanogaster males can be made from this generalised model. However, these predictions depend upon whether one is concerned with differences in courtship behaviour between males or with differences in time in the courtship behaviour of single males.

Comparisons between males

These predictions depend completely upon the distribution of mean M in the particular population of males. For example, if all the males in a population have their mean M below To, then the relationships between males are: proportion courtship positively correlated with proportion orientation, proportion courtship positively correlated with proportion vibration, proportion courtship postively correlated with rate of licking and so, therefore, proportion orientation positively correlated with proportion vibration and proportion vibration positively correlated with rate of licking (all proportions of time). Table 1 specifies the relationships in the

Table 1. Correlation of courtship behaviour patterns between males

Behaviour patterns	Correlation		
	M < Mo	Mo < M < Mv	Mv < M
courtship - orientation	+	-	-
courtship - vibration	+	+	-
courtship - licking	+	+	+
courtship - att. copulation	+	+	+
orientation - vibration	+	-	-
orientation - licking	+	-	-
orientation - att. copulation	+	-	-
vibration - licking	+	+	-
vibration - att. copulation	+	+	-
licking - att. copulation	+	+	+

other simple cases in which all males are grouped in the same region of M in terms of thresholds. It is obvious that if the mean levels of M of the males are dispersed over two or more regions the relationships become so diverse as to be unpredictable and of no use in testing the model.

Comparisons within males over time

The possibilities in this category are strictly constrained. If a trend with time occurs in any behaviour then trends must be present in all behaviours as they all are determined by the same underlying variable M. Furthermore, the specific directions of the trends in all the behaviour patterns as a whole are limited. The only possible combinations (for simple linear regressions) are set out in Table 2, and correspond to a subset of the possible combinations for comparisons across males. If the trends present in some behaviour patterns are curvilinear or unstable with time then the trends in all the other behaviour patterns must be affected similarly.

Although the model does not explicitly predict boutlengths, trends in both boutlengths and frequencies of occurrence can be used to test the model. For example, the probability of vibration will increase with time under any of the following conditions,

1) boutlengths constant, frequency increases

2) boutlengths increase, frequency constant

3) boutlengths increase, frequency increases

4) rate of boutlength increase > rate of frequency decrease

5) rate of frequency increase > rate of boutlength decrease.

Many courtships will be found to have significant trends in some courtship elements but not in all. Unfortunately, statistically, the absence of a trend can never be proved; only a maximum and a minimum

Table 2. Patterns of change in the probability of acts with time within males (+ = increase, - = decrease).

Courtship	Orientation	Vibration	Lick	Att. copulation	Level of M
+	+	+	+	+	$<M_O$
+	-	+	+	+	$>M_O$ $<M_V$
+	-	-	+	+	$>M_V$
-	-	-	-	-	$<M_O$
-	+	-	-	-	$>M_O$ $<M_V$
-	+	+	-	-	$>M_V$

limit to the magnitude of the trend can be determined. These limits can be narrowed by increasing the sample size. In practice this is difficult if not impossible with Drosophila since quasinormal courtships ending in copulation are not very long and the trends during very long non-copulatory courtships (i. e. with unreceptive females) will not be simple. However, any hypothesis which states that trends are present in all courtship acts but are so small in some behaviours that they cannot be shown to be statistically significant is not a scientific hypothesis; it is not disprovable (Popper, 1959), and is not acceptable unless the hypothesis sets a lower value for the magnitude of trends. In this work non-significance of trends at the 5% probability level will mean acceptance of (but not proof of) the null hypothesis of no trend for the particular behaviour element.

Chapter 4. Temporal patterning of courtship behaviour

The sequence of behavioural events during courtship can be resolved into two components, the duration of each behavioural element (bout duration) and the interval between two bouts of the same element (bout interval). Since all of the behaviour patterns may be repeated during a courtship it is important to examine whether these two measures change with time.

Method

Trends may exist in bout durations or bout intervals or both during the course of a courtship. The presence of temporal patterning in a specific behavioural act is examined with the statistical technique of regression (Snedecor and Cochran, 1967). Trends may be linear, curvilinear (e.g. polynomial - quadratic), cyclical or periodic, etc.

In this chapter three types of trends will be fitted to the bout durations and to the bout intervals.

1) Linear - the equation for this form of trend is $y = a + bx$ or $y = bx$ where y is the bout duration or interval (measured in log sec); x is time in seconds, b is the slope or linear coefficient and a is the y-intercept or elevation or position or adjusted mean (i.e. a is the bout duration when x (time) is 0 sec.

2) Quadratic - this type of trend may have the form $y = a + bx + cx^2$ or the form $y = a + cx^2$ or the form $y = cx^2$, where c is the quadratic coefficient and the other terms are the same as for the linear equation.

3) Cyclical - the simplest cyclical trend is a sine or cosine wave. A sine wave is approximated, to any desired degree of accuracy, by the Taylor series, $\sin x = x - x^3/3! + x^5/5! - x^7/7! + \ldots$ (i.e. a

polynomial in odd powers of x, Aleksandrov et al., 1963).

These trends can be fitted to each courtship separately. In any regression analysis there are three questions that can be asked of the data.

1) Do all courtships have the same y-intercepts?

2) Are the coefficients different from zero?

3) Are the coefficients the same in all the courtships?

These questions are answered by an analysis of covariance.

Directional trends

Under the term directional I include linear and quadratic trends.

Orientation

Table 3 (Fig. 4) shows the results of an analysis of covariance of orientation bout durations. Orientation bouts decreased in duration during courtship (significant negative linear coefficient) and the rate of decrease increased with time (significant negative quadratic coefficient). All males had the same slopes but the males' elevations differed significantly. As indicated in Table 4 and Fig. 5 orientation intervals decreased with time (significant negative linear coefficient) but the rate of decrease levelled off with time (significant positive quadratic coefficient). For this measure the males differed in both their coefficients and their y-intercepts.

Vibration

Vibration bout durations showed no change with time during courtship. The males did differ in their mean vibration bout durations which due to the lack of trends were their elevations

Table 3. Analysis of covariance of ORI bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.99	5.59	0.0010
Linear coefficient	1	1.96	11.10	0.0010
Quadratic coefficient	1	1.58	8.94	0.0010
Divergence in slopes	208	0.20	1.12	NS
Between positions	104	0.34	1.94	0.0010
Error	985	0.18		

Linear coefficient = 0.0031

Y-intercept = -0.0707

Quadratic coefficient = -0.000014

Figure 4. Overall regression of ORI bouts (H) on duration of courtship (T).

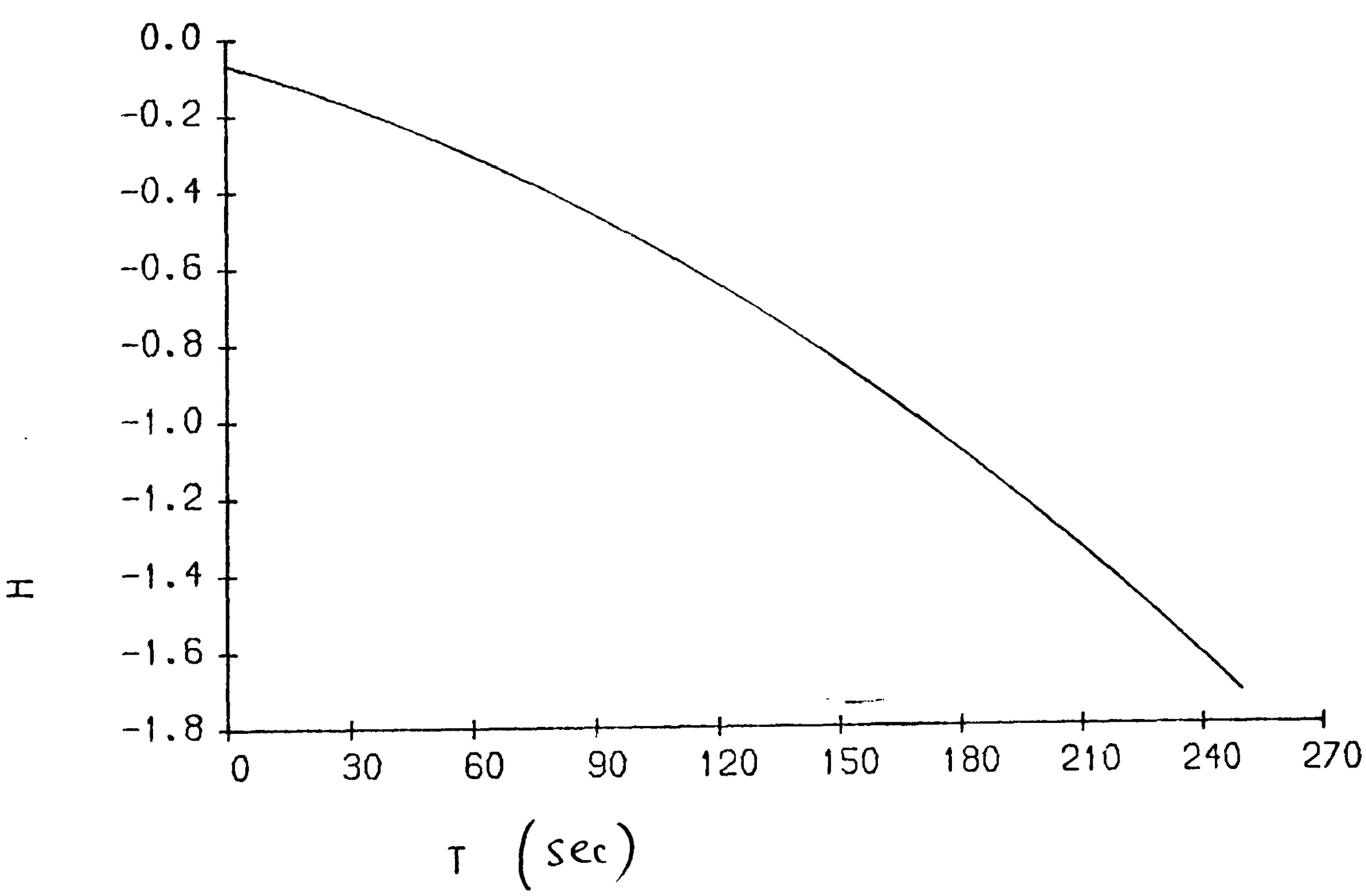


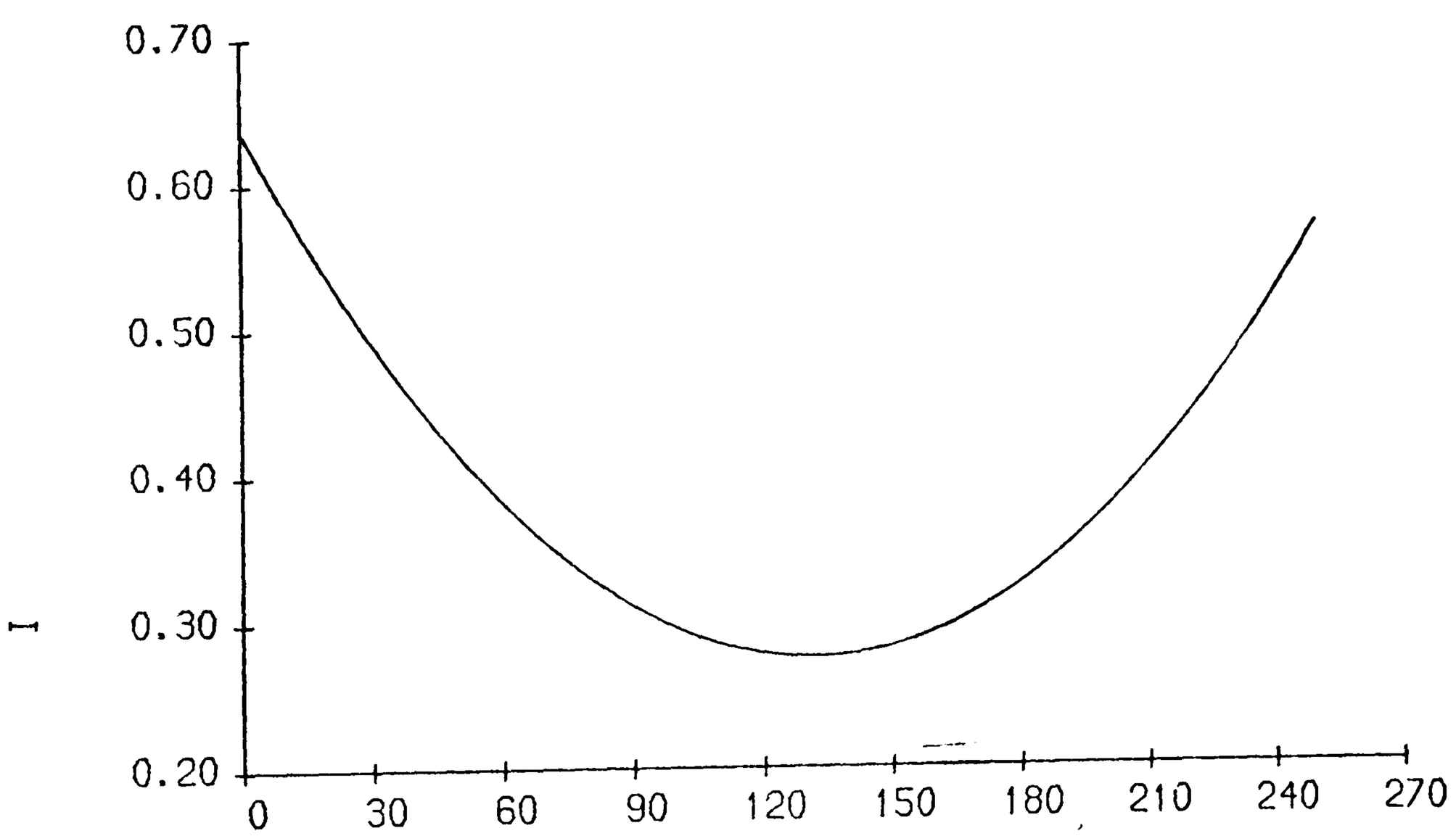
Table 4. Analysis of covariance of ORI intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	3.43	23.27	0.0010
Linear coefficient	1	5.42	36.78	0.0010
Quadratic coefficient	1	2.96	20.04	0.0010
Divergence in slopes	186	0.21	1.46	0.0010
Between positions	93	0.31	2.13	0.0010
Error	880	0.15		

Linear coefficient = -0.0055

Y-intercept = 0.6370

Figure 5. Overall regression of ORI intervals on time. T = duration of courtship (sec); I = duration of interval (log sec).



(Table 5). The intervals between vibration bouts decreased linearly with time (Fig. 6). The quadratic coefficient was not significantly different from zero and is not included in Table 6. Males differed in both their coefficients and their y-intercepts.

Lick

Since licks have a relatively constant and very short duration (< 0.2 sec) only intervals can be analysed for trends (Table 7, Fig. 7). The intervals between licks decrease linearly during courtship and all males had the same linear coefficient. However, the males did differ significantly in their elevations.

Attempted copulation

Occasionally the male will attempt to copulate by mounting the female and remaining on her back for an appreciable length of time. During this time the male may repeatedly attempt to engage his genitalia with the female's genitalia or he may remain still. The duration of these mounts decreased during courtship and males were similar in both their coefficients and their y-intercepts (Table 8, Fig. 8). There were no temporal trends in the intervals between copulation attempts nor did the males differ in their elevations (Table 9).

Non-courtship

Table 10 indicates that the duration of non-courtship bouts decreased linearly with time and that males differed in their rates of decrease and in their y-intercepts (Fig. 9). Table 11 indicates that the trends in non-courtship intervals differ between males but that the overall coefficients were not significantly different from

Table 5. Analysis of covariance of VIB bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.02	0.18	NS
Linear coefficient	1	0.04	0.36	NS
Quadratic coefficient	1	0.04	0.31	NS
Divergence in slopes	184	0.11	0.98	NS
Between positions	92	0.19	1.59	NS
Error	856	0.12		

Table 6. Analysis of covariance of VIB intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	2.66	10.25	0.0100
Linear coefficient	1	0.53	2.03	NS
Quadratic coefficient	1	0.00	0.01	NS
Divergence in slopes	162	0.30	1.14	NS
Between positions	81	0.67	2.59	0.0100
Error	763	0.26		

Linear coefficient = -0.0022

Y-intercept = 0.4511

Figure 6. Overall regression of VIB intervals on time. T = duration of courtship (sec); J = duration of interval (log sec)

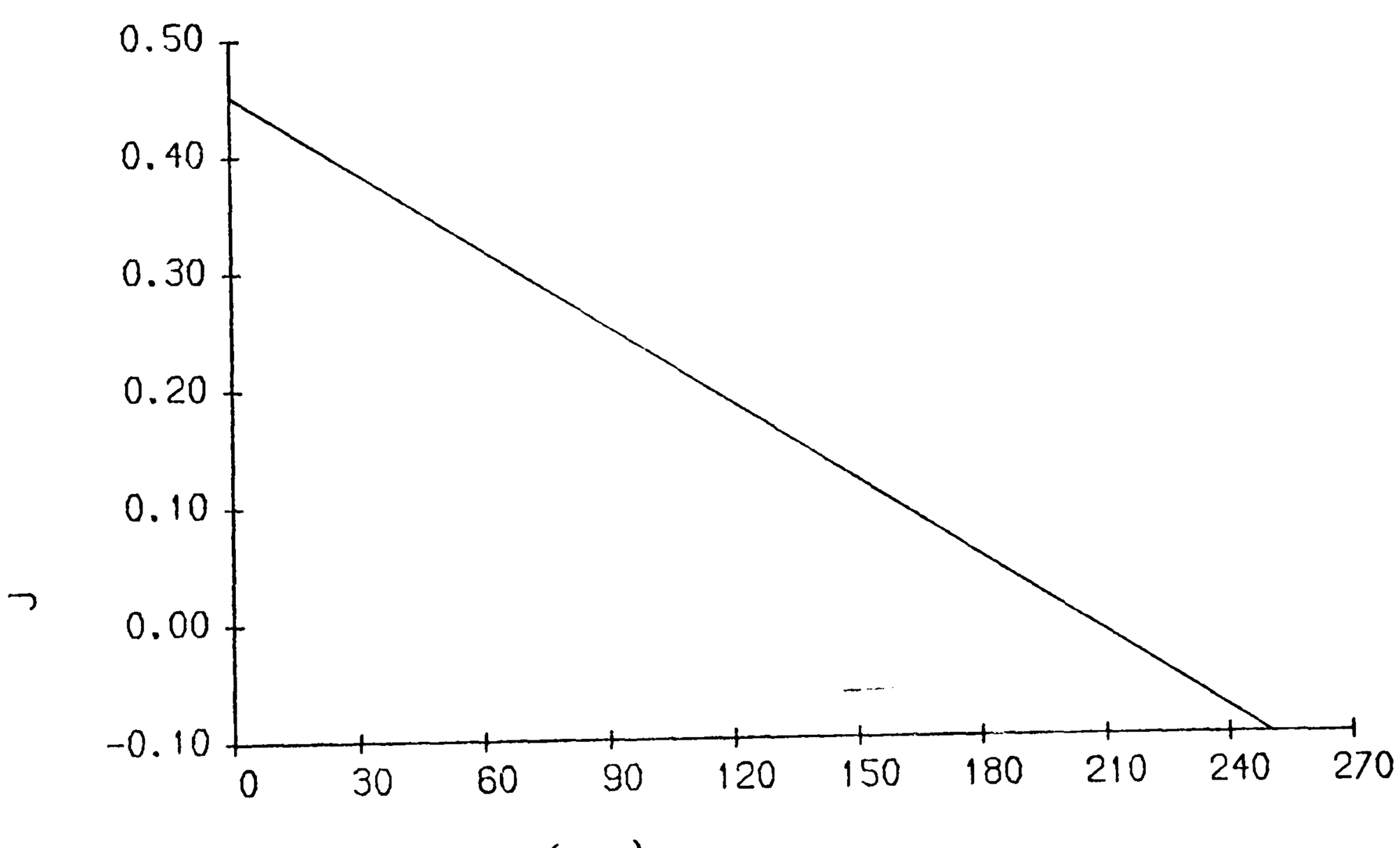


Table 7. Analysis of covariance of LIC intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.91	6.75	0.0010
Linear coefficient	1	0.01	0.06	NS
Quadratic coefficient	1	0.24	1.78	NS
Divergence in slopes	50	0.09	0.66	NS
Between positions	25	0.50	3.68	0.0100
Error	123	0.13		

Linear coefficient = -0.0022

Y-intercept = 1.3991

Figure 7. Overall regression of LIC intervals (K) on the duration of courtship (T).

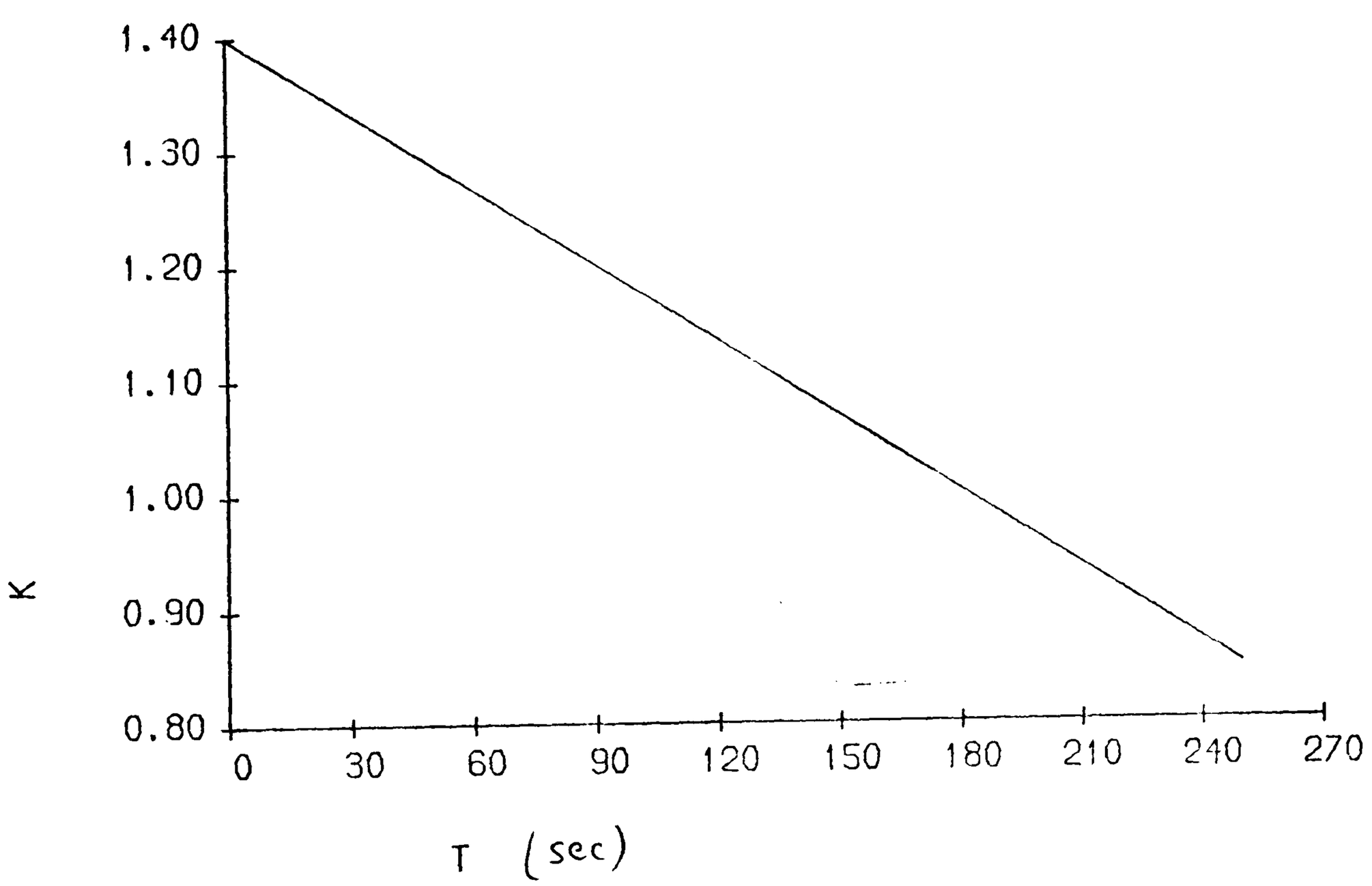


Table 8. Analysis of covariance of ATT bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	1.85	14.43	0.0010
Linear coefficient	1	1.72	13.35	0.0010
Quadratic coefficient	1	0.77	6.02	0.0010
Divergence in slopes	40	0.14	1.10	NS
Between positions	20	0.33	2.58	NS
Error	66	0.13		

Linear coefficient = -0.0069

Y-intercept = 0.0165

Figure 8. Overall regression of ATT bouts (L) on the duration of courtship (T).

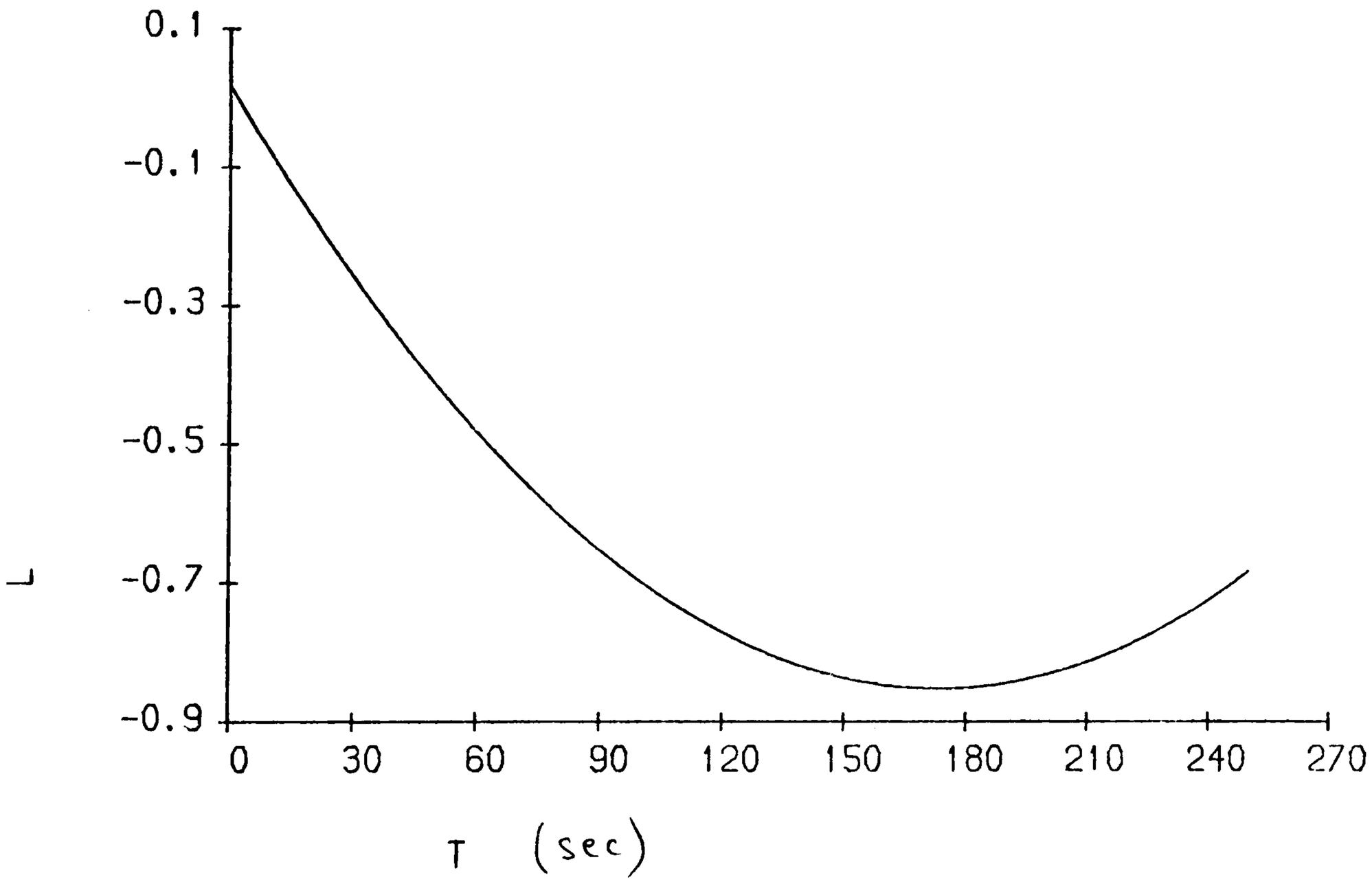


Table 9. Analysis of covariance of ATT intervals on time

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.15	1.02	NS
Linear coefficient	1	0.26	1.77	NS
Quadratic coefficient	1	0.30	2.02	NS
Divergence in slopes	28	0.21	1.43	NS
Between positions	14	0.31	2.08	0.0500
Error	45	0.15		

Table 10. Analysis of covariance of NON bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.45	2.91	NS
Linear coefficient	1	0.11	0.70	NS
Quadratic coefficient	1	0.00	0.00	NS
Divergence in slopes	72	0.23	1.50	0.0500
Between positions	36	0.30	1.97	0.0500
Error	141	0.15		
Linear coefficient =	-0.0069			
Y-intercept =	0.0165			

Figure 9. Overall regression of NON bouts (M) on the duration of courtship (T).

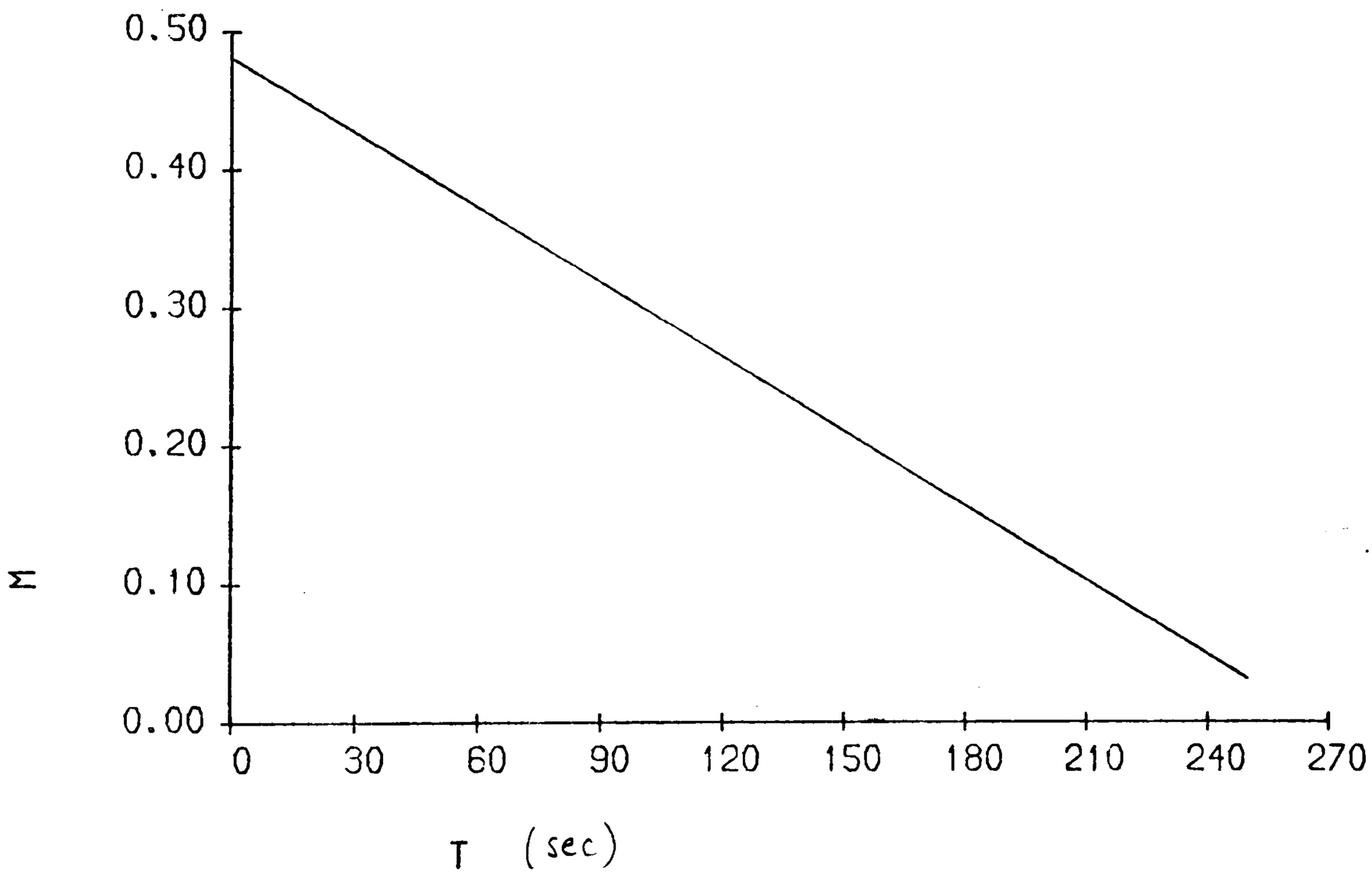


Table 11. Analysis of covariance of NON intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.16	0.60	NS
Linear coefficient	1	0.30	1.17	NS
Quadratic coefficient	1	0.30	1.17	NS
Divergence in slopes	50	0.31	1.20	NS
Between positions	25	0.49	1.91	0.0500
Error	104	0.26		

zero (i.e. there were significant positive coefficients in some males and negative coefficients in others).

Run

Run bout durations decreased linearly during courtship and females differed only in their elevations (Table 12, Fig. 10). The intervals between run bouts increased in duration and females differed both in their coefficients of increase and their y-intercepts (Table 13, Fig. 11).

Stand

Since standing is the converse of running, the results of the analyses of covariance should be the converse of those for running, except that the first interval of each is not included in the bout analyses of the converse behaviour. Therefore no separate analysis is included for this behaviour.

Fend

There were no trends in the bout durations of fending or in the intervals between fends (data not shown). Due to the small amount of data available for this behaviour no further analyses of fending will be undertaken in this thesis.

Elevation

The duration of elevation bouts decreased linearly with time (Table 14, Fig. 12). The coefficients and the y-intercepts differed between females. The intervals between elevations also decreased linearly with time and females differed only in their y-intercepts for this measure (Table 15, Fig. 13).

Table 12. Analysis of covariance of RUN bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	2.67	11.01	0.0500
Linear coefficient	1	2.06	8.52	0.0500
Quadratic coefficient	1	0.32	1.31	NS
Divergence in slopes	186	0.33	1.35	0.0500
Between positions	93	0.45	1.85	0.0100
Error	560	0.24		
Linear coefficient =	-0.0023			
Y-intercept =	0.7590			

Figure 10. Overall regression of RUN bouts (G) on the duration of courtship (T).

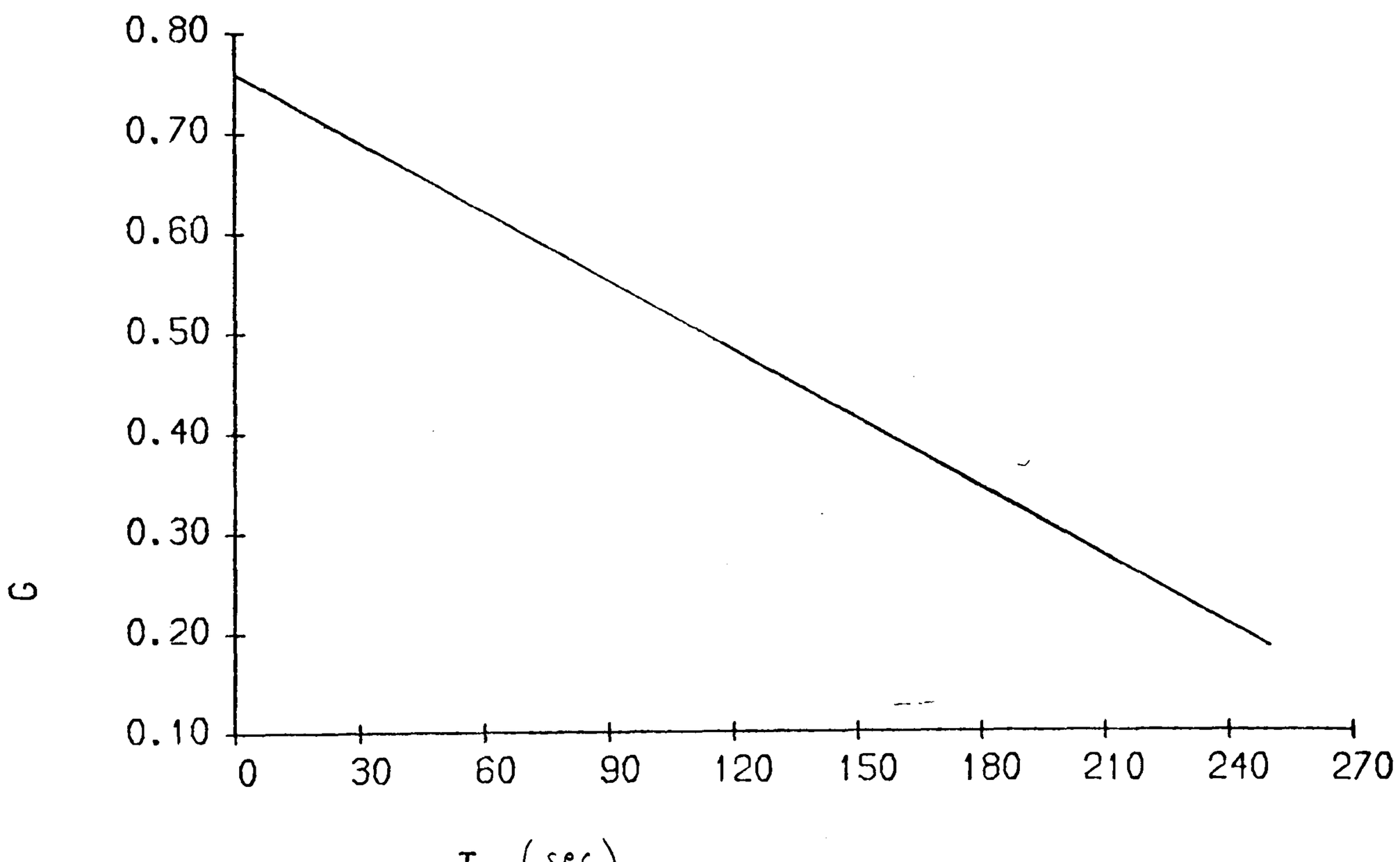


Table 13. Analysis of covariance of RUN intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.51	3.86	0.0500
Linear coefficient	1	0.37	2.80	NS
Quadratic coefficient	1	0.05	0.37	NS
Divergence in slopes	150	0.26	1.99	0.0100
Between positions	75	0.35	2.65	0.0100
Error	464	0.13		

Linear coefficient = 0.0016

Figure 11. Overall regression of RUN intervals (A) on the duration of courtship (T).

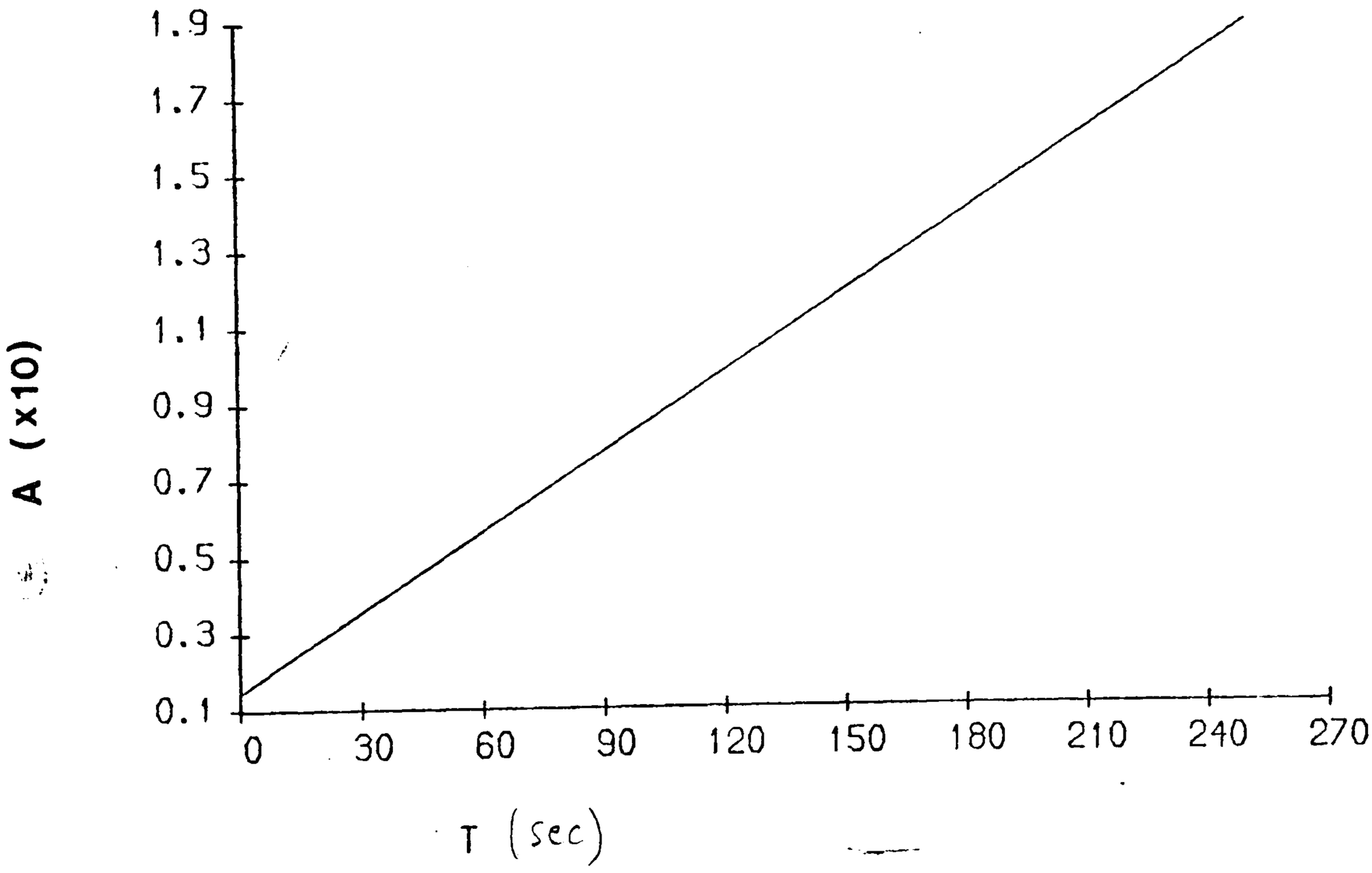


Table 14. Analysis of covariance of ELE bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.40	5.36	0.0100
Linear coefficient	1	0.21	2.74	NS
Quadratic coefficient	1	0.04	0.47	NS
Divergence in slopes	50	0.12	1.66	0.0500
Between positions	25	0.33	4.38	0.0500
Error	99	0.08		

Linear coefficient = -0.0018

Y-intercept = 0.5280

Figure 12. Overall regression of ELE bouts (E) on the duration of courtship (T).

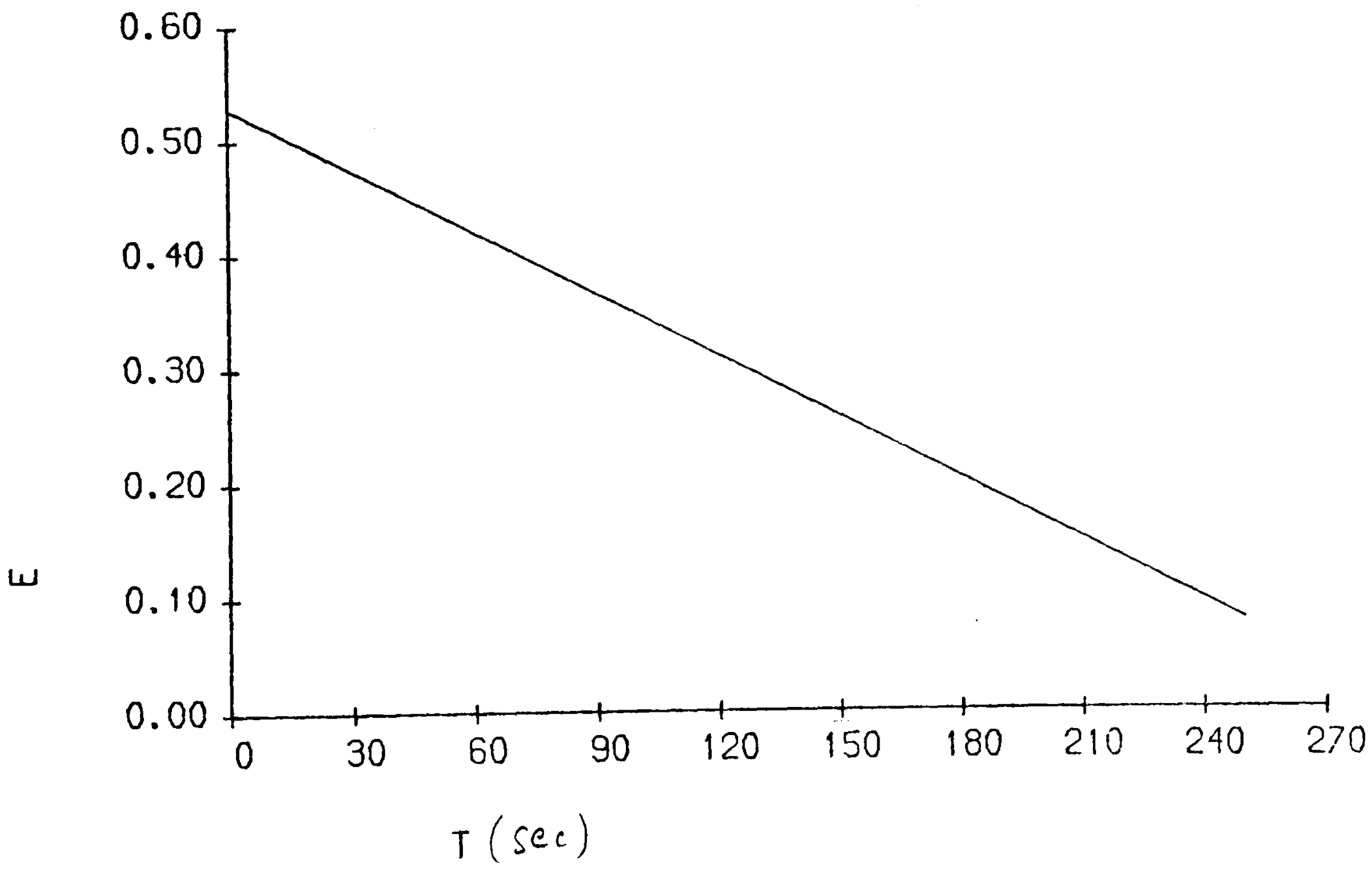


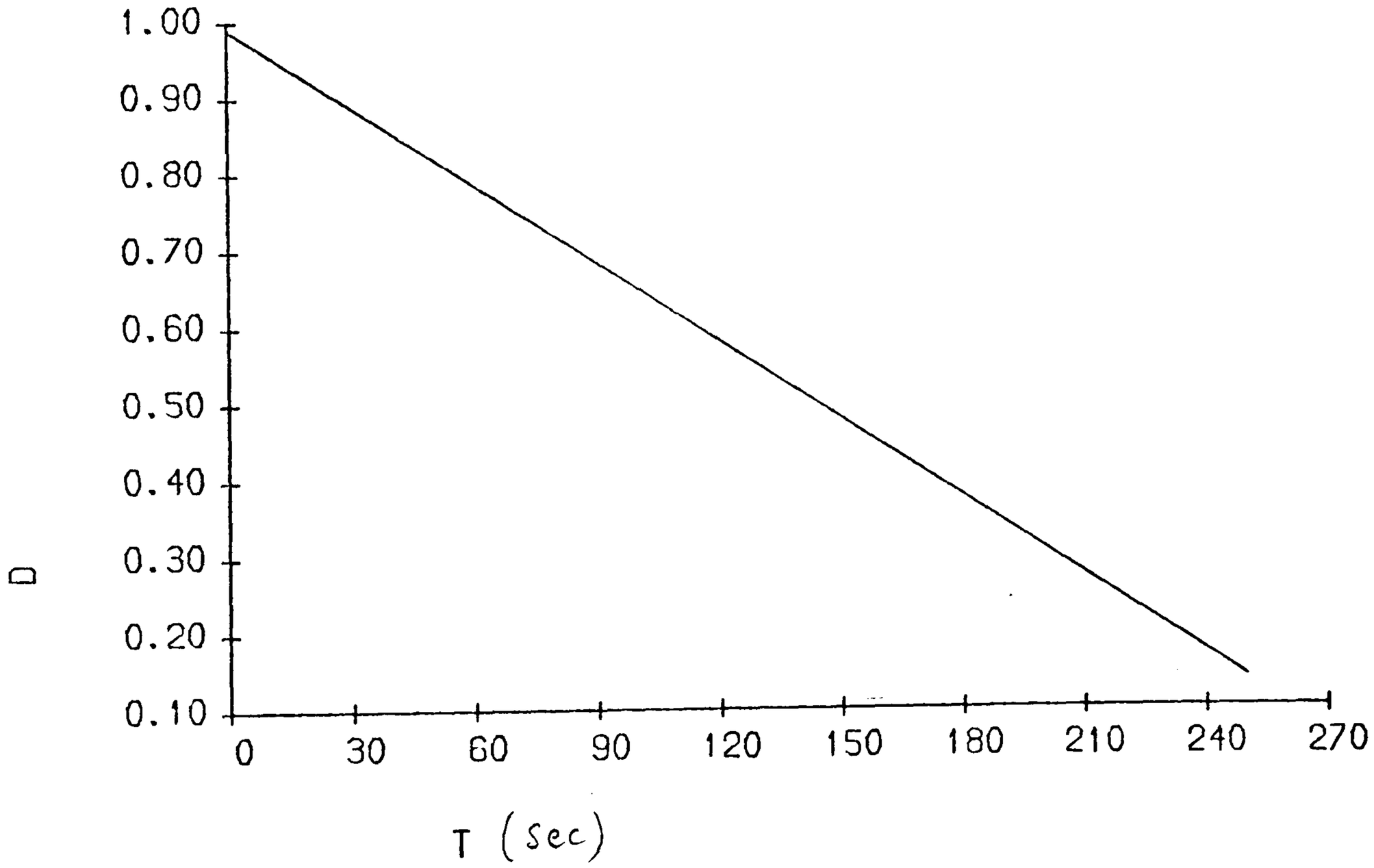
Table 15. Analysis of covariance of ELE intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	1.13	9.83	0.0500
Linear coefficient	1	0.00	0.02	NS
Quadratic coefficient	1	0.18	1.53	NS
Divergence in slopes	30	0.21	1.82	0.0500
Between positions	15	0.43	3.72	0.0500
Error	73	0.11		

Linear coefficient = -0.0034

Y-intercept = 0.9892

Figure 13. Overall regression of ELE intervals (D) on the duration of courtship (T).



Flick

There was insufficient data for the analysis of flicks and no further analysis of this behaviour will be attempted in this thesis.

Kick

The duration of kick bouts decreased with time (Table 16). Females did not differ in their coefficients or in their elevations. The intervals between kicks also decreased with time. Females did not have different coefficients but did differ in their elevations (Table 17, Fig. 14).

Extrusions

There were no temporal trends in either bout durations or intervals for this behavior (Tables 18, 19). Nor did the females differ significantly in their means.

Preening

Table 20 indicates that the duration of preening bouts increased during courtship (Fig. 15) and that females differed significantly in their y-intercepts. The intervals between preening bouts decreased with time and females again differed only in their y-intercepts (Table 21, Fig. 16).

Discussion

In the male the overall conclusion we may draw is that the 'tempo' of the courtship increases with time, with more time spent courting and less spent non-courting. The bout durations of orientation increase while those of non-courtship decrease with time. The

Table 16. Analysis of covariance of KIC bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.14	6.59	0.0050
Linear coefficient	1	0.13	6.26	0.0050
Quadratic coefficient	1	0.20	9.62	0.0050
Divergence in slopes	18	00.02	0.78	NS
Between positions	9	0.01	0.48	NS
Error	47	0.02		

Linear coefficient = -0.0039

Quadratic coefficient = 0.0000

Y-intercept = -0.7070

Table 17. Analysis of covariance of KIC intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.37	4.21	0.0500
Linear coefficient	1	0.08	0.93	NS
Quadratic coefficient	1	0.24	2.72	NS
Divergence in slopes	14	0.25	2.82	0.0100
Between positions	7	0.73	8.25	0.0100
Error	37	0.09		

Linear coefficient = -0.0024

Y-intercept = 1.2130

Figure 14. Overall regression of KIC interval (C) on the duration of courtship (T).

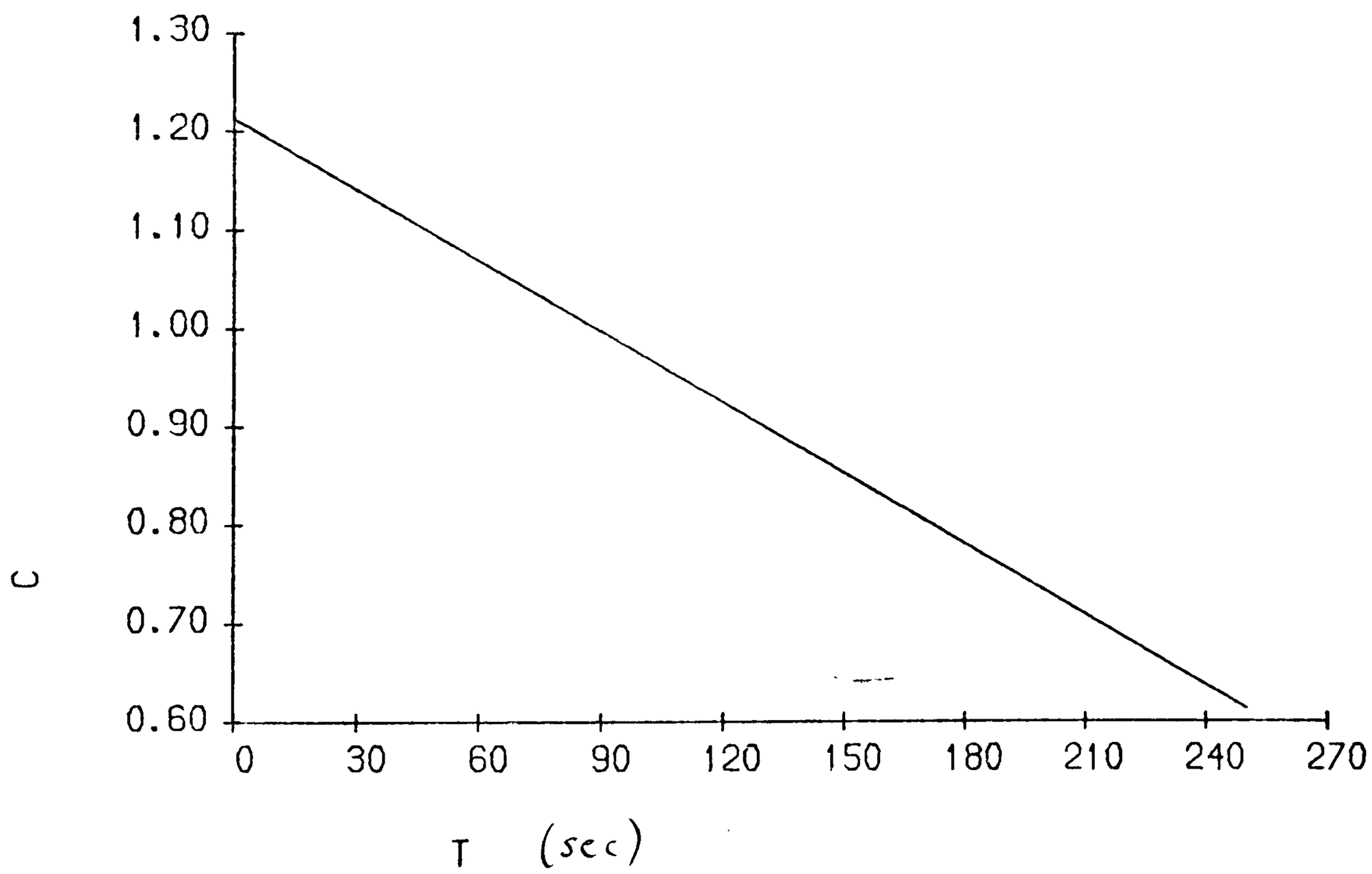


Table 18. Analysis of covariance of EXT bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.00	0.03	NS
Linear coefficient	1	0.01	0.06	NS
Quadratic coefficient	1	0.01	0.06	NS
Divergence in slopes	16	0.31	3.20	0.0500
Between positions	8	0.13	1.36	NS
Error	18	0.10		

Table 19. Analysis of covariance of EXT intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.30	1.79	NS
Linear coefficient	1	0.60	3.57	NS
Quadratic coefficient	1	0.54	3.20	NS
Divergence in slopes	12	0.10	0.62	NS
Between positions	6	0.32	1.88	NS
Error	9	0.17		

Table 20. Analysis of covariance of PRE bouts on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.45	3.90	0.0500
Linear coefficient	1	0.89	7.74	0.0100
Quadratic coefficient	1	0.76	6.61	0.0500
Divergence in slopes	20	0.14	1.19	NS
Between positions	10	0.39	3.44	0.0500
Error	38	0.11		
Linear coefficient =	0.0097			
Y-intercept =	-0.7386			

Figure 15. Overall regression of PRE bouts (B) on the duration of courtship (T).

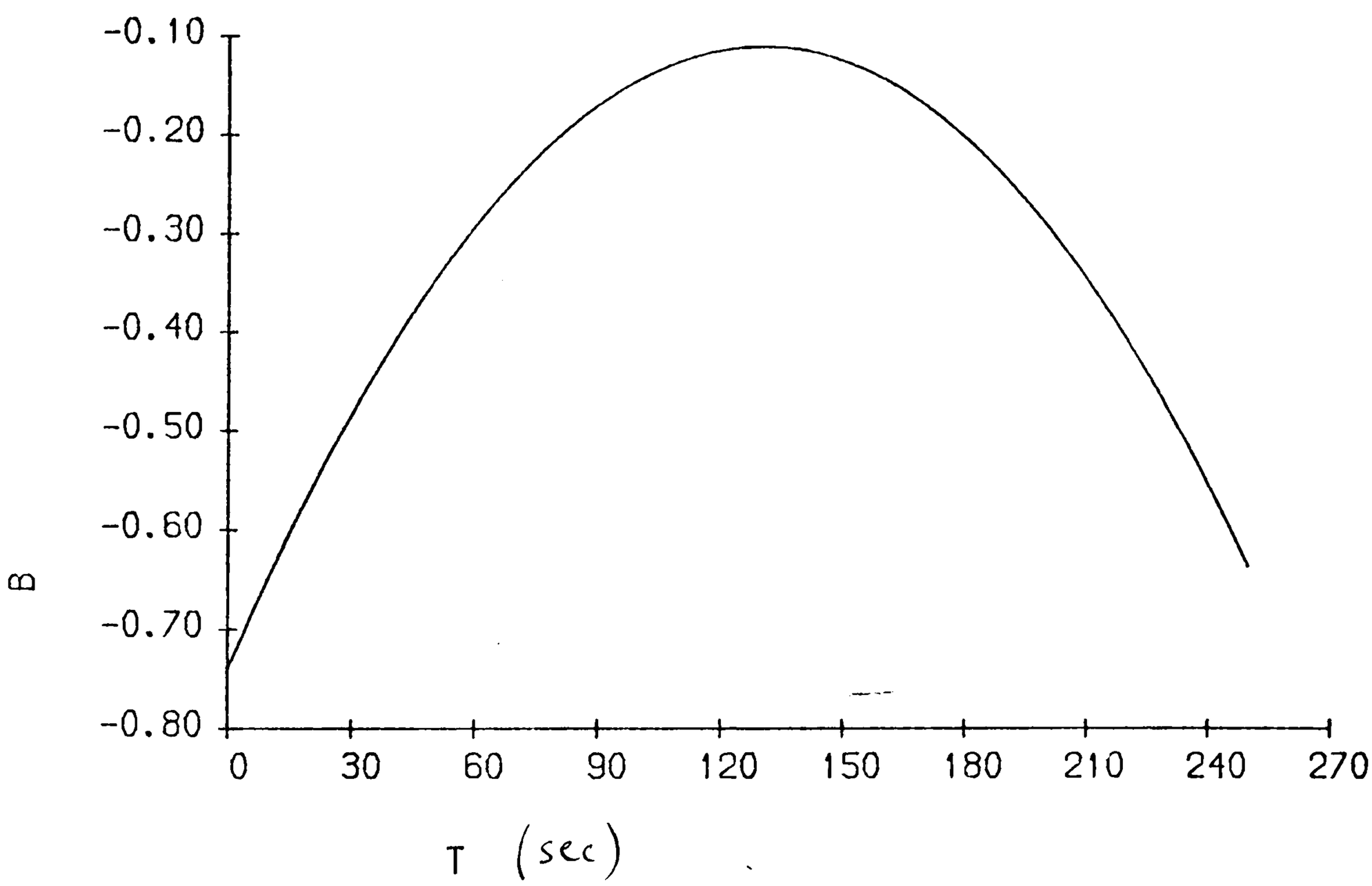


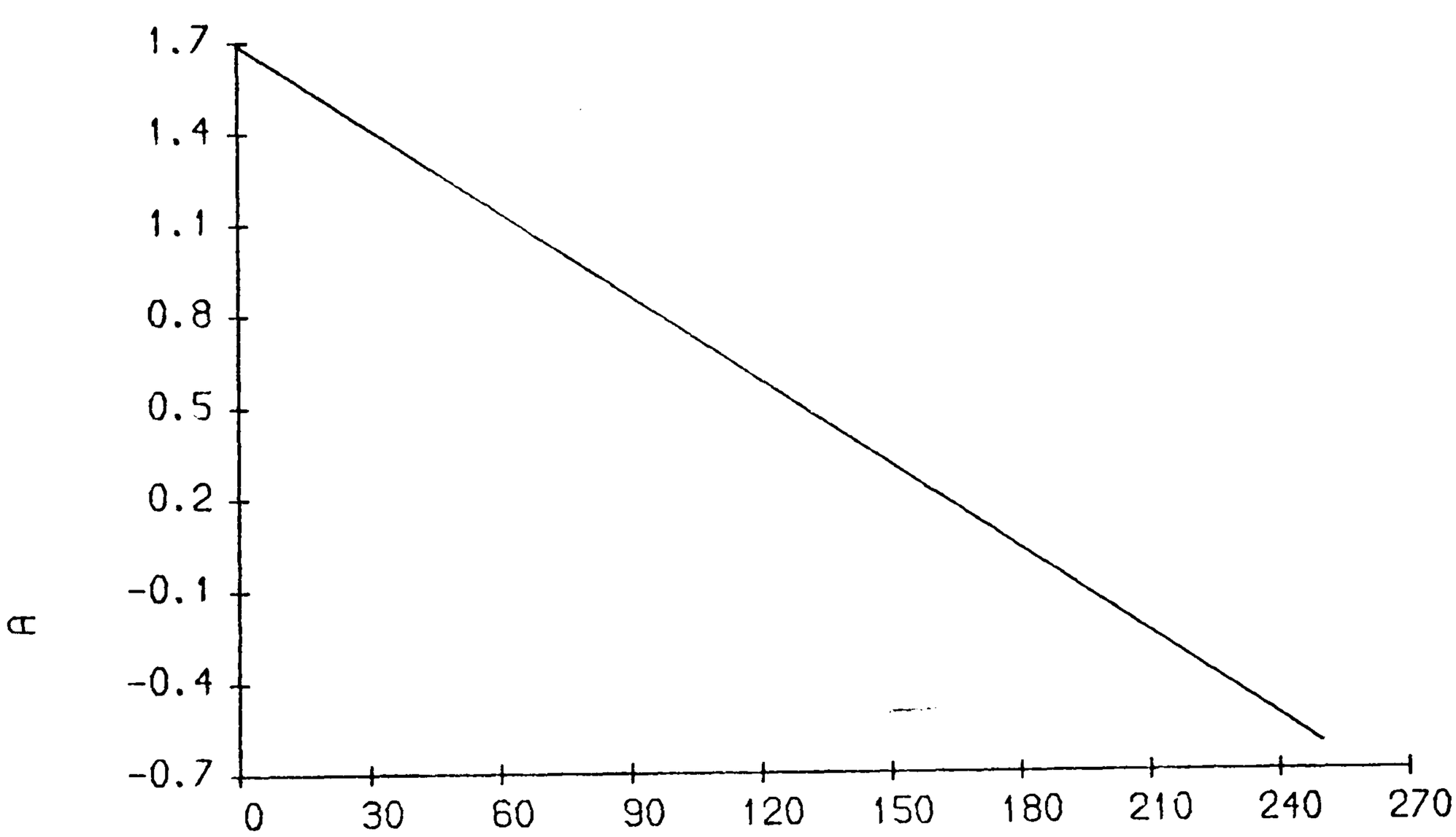
Table 21. Analysis of covariance of PRE intervals on time.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	2.50	12.48	0.0100
Linear coefficient	1	0.39	1.96	NS
Quadratic coefficient	1	0.01	0.06	NS
Divergence in slopes	14	0.25	1.27	NS
Between positions	7	0.67	3.33	0.0500
Error	27	0.20		

Linear coefficient = -0.0092

Y-intercept = 1.6892

Figure 16. Overall regression of PRE intervals on time. T = duration of courtship (sec); A = duration of interval.



intervals between behavioural elements decrease in duration for orientation, vibration and licking. Thus these behaviours increase in frequency during courtship. The frequency of attempted copulations remains stable while that of non-courtship bouts decreases with time.

At first sight there would appear to be an incremental process affecting the courtship behaviour of male D. melanogaster (Hinde, 1970). But does the stimulus for courtship, the female, remain constant? It does not. Among other changes, the female stands more often and for longer periods of time as the courtship progresses. The increased frequency of the male courtship elements might be a simple physical consequence of this.

The original model for the courtship behaviour of D. melanogaster (Bastock and Manning, 1955; Bastock, 1956) predicts that orientation bout durations would be negatively correlated with vibration bout durations. There can be no significant correlation in this set of data as orientation bouts increase in duration with time but vibration bouts do not change in duration during courtship. Their model also predicts that the duration of vibration bouts would be positively correlated with the frequency of licks. Again, there can be no significant correlation between vibration bout durations which do not change with time and lick frequency which increases with time during courtship. The temporal patterning of male courtship behaviour does not support Bastock and Manning's threshold model.

The female spends more time standing and stands more frequently as courtship proceeds. This confirms the findings of Cook (1973), and of Schilcher (1976) who found that courtship song caused females to spend less time running. However, in these experiments the females may have been disturbed when the partition was removed from the observation cells at the start of the videotaping. The decline in

running may have been due only to the gradual settling down of the female to her spontaneous activity level (Connolly, 1967).

There is no evidence from this data that fending is related to any male courtship behaviour pattern, except possibly attempted copulation. The results of the analysis of covariance for extrusion intervals was similar to that for attempted copulation intervals and a relationship between extrusion and attempted copulation is plausible due to a similar finding by Connolly and Cook (1973). Both elevations and kicks decrease in duration but increase in frequency with time. This suggests that these behaviours may be related to licks which also increase in frequency with time. Since females can preen only when standing still, the trends in preening may be a simple and direct consequence of those in standing. However, if preening is due to the female being disturbed by contact with the male during either licks or attempted copulations or both, this may also explain the trends in preening.

Cyclical trends

Table 22 indicates that there were significant cyclical trends in orientation bout durations. Males differed in their coefficients and in their y-intercepts. There were no significant cycles in vibration bout durations (Table 23). The duration of non-courtship bouts cycled with significantly different coefficients in different males. Males had significantly different elevations but the overall coefficients were not significantly different from zero (Table 24). There were significant cycles in orientation intervals. Males differed in both the elevations and coefficients of these cycles (Table 25). Cycles did not occur in vibration intervals (Table 26). There

Table 22. Cycles in ORI bouts with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	61	0.44	2.44	0.0000
Zero slope	4	1.06	5.87	0.0001
Error	981	0.18		
Equality of slopes	244	0.22	1.29	0.0060
Error	737	0.17		

Linear coefficient = 0.5819 ± 0.1256

Cubic coefficient = -0.5498 ± 0.1405

Quintic coefficient = 0.1962 ± 0.0557

Seventh power coeff.= -0.0219 ± 0.0066

The presence of significant cycles is indicated by the statistical significance of the 'Zero slope' term in the analysis of covariance and by the significance of the coefficients which are given with their standard errors. Coefficients are significant if at least twice as large as their standard errors.

This applies to the following tables.

Table 23. Cycles in VIB bouts with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	63	0.21	1.78	0.0003
Zero slope	3	0.07	0.62	0.6000
Error	928	0.12		
Equality of slopes	189	0.11	0.89	0.8400
Error	739	0.12		

Table 24. Cycles in NON bouts with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	10	0.39	2.31	0.0167
Zero slope	3	0.12	0.72	0.5400
Error	110	0.17		
Equality of slopes	30	0.31	2.67	0.0003
Error	80	0.12		

Table 25. Cycles in ORI intervals with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	59	0.36	2.38	0.0000
Zero slope	3	1.64	10.75	0.0000
Error	922	0.15		
Equality of slopes	177	0.20	1.40	0.0015
Error	745	0.14		

Linear coefficient = -0.4348 ± 0.0891

Cubic coefficient = 0.1516 ± 0.0554

Quintic coefficient = -0.019 ± 0.0099

Table 26. Cycles in VIB intervals with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	54	0.74	2.74	0.0000
Zero slope	3	1.47	5.47	0.0010
Error	809	0.27		
Equality of slopes	162	0.28	1.03	0.4000
Error	647	0.27		

were no cycles in lick intervals (Table 27), or in non-courtship intervals (Table 28). There were significant cycles in run bout durations. The coefficients for these cycles were similar in all females. However, females differed in their y-intercepts (Table 29). Females differed in the coefficients of their run interval cycles and in their y-intercepts (Table 30). However, the overall coefficients were not significantly different from zero.

Discussion

It is clear that the temporal patterning of courtship behaviour in D. melanogaster is not simple. The presence of cycles in both male and female courtship patterns raises the question of what causes the cycles. Are the cycles inherent in male behaviour and the female cycles correlates of these, or vice versa, or are the male and female cycles independent, or are they both linked to some external environmental variable?

In this section I have used Taylor series to detect cyclical trends. Since these series are polynomials it is difficult to compare cycles in males with those in their own females (there may be more than one significant power coefficient and this leads to multiple correlation analysis). To do this efficiently one should submit courtship data to Fourier analysis (Bliss, 1970) and estimate amplitudes and phase angles. These can then be correlated between males and females to determine which sex leads the other (if either). One needs much longer than normal courtships for this method of analysis. The results suggest that the cycles in male courtship behaviour and in running in the female are independent. All females followed the same cycle in the duration of their running bouts but the males had different cycles in their orientation bouts and in

Table 27. Cycles in LIC intervals with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	7	0.81	6.06	0.0000
Zero slope	1	1.89	14.13	0.0003
Error	100	0.13		
Equality of slopes	7	0.10	0.73	0.6460
Error	93	0.14		

Linear coefficient = -0.2853 ± 0.0759

Table 28. Cycles in NON intervals with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	7	0.48	2.07	0.0600
Zero slope	3	0.21	0.89	0.4500
Error	81	0.23		
Equality of slopes	21	0.30	1.43	0.1400
Error	60	0.21		

Table 29. Cycles in RUN bouts with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	46	0.44	1.73	0.0026
Zero slope	4	1.12	4.45	0.0015
Error	555	0.25		
Equality of slopes	184	0.27	1.12	0.1749
Error	371	0.24		

Linear coefficient = -0.5455 ± 0.1724

Cubic coefficient = 0.5864 ± 0.2368

Quintic coefficient = -0.2258 ± 0.1043

Seventh power coeff. = 0.0245 ± 0.0135

Table 30. Cycles in RUN intervals with time.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	43	0.37	2.16	0.0001
Zero slope	3	0.36	2.05	0.1057
Error	481	0.17		
Equality of slopes	129	0.30	2.40	0.0000
Error	352	0.13		

their non-courtship bouts. However, definite answers to the above questions must await further experimentation explicitly designed for the study of cycles.

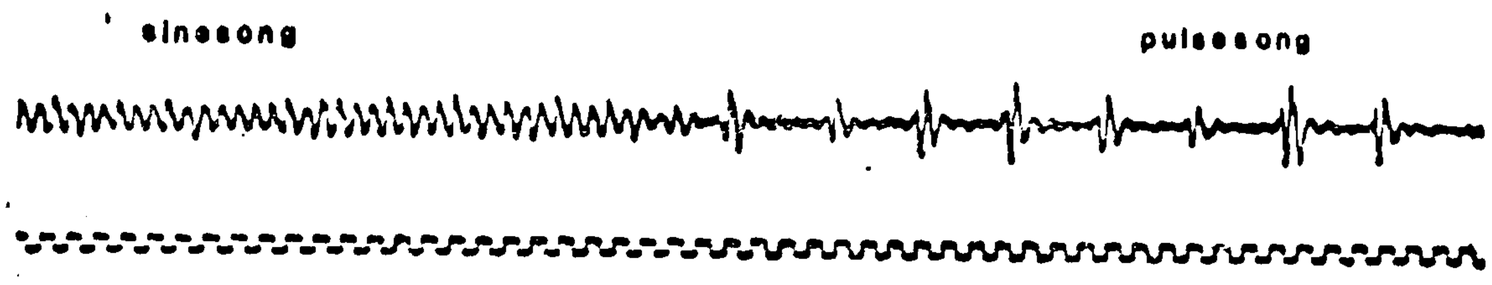
Chapter 5. Temporal patterning of courtship song

Introduction

In the previous chapter we saw that there were no trends in the duration of vibration bouts. However, two types of sound are produced during wing vibration and these may show trends even though vibration bouts do not. These have been named pulse song and sine song (see Fig. 17) and the effects of these songs on both male and female behaviour have been investigated by Schilcher (1976a, b). He found that when a male heard another male's song, it responded with an increase in locomotor and courtship activity. Both sine song and pulse song reduced the locomotor activity of sexually mature females, and both songs stimulated females to mate. However, only the stimulation of mating speed produced by sine song was stored (i.e. was evident if the males were introduced to the females some time after the sound was switched off) and - possibly - summated by the female. The inter-pulse-interval (ipi) of pulse song (see Fig. 18) has been shown to be an important parameter in determining the stimulatory value of pulse song (Bennet-Clark and Ewing, 1969). Ipi differs between sibling species (for D. melanogaster the mean ipi at 25° C is 34.6 msec and for D. simulans it is 48 msec) and it has been suggested that pulse song acts as a species identifier (Bennet-Clark and Ewing, 1969). For the pulse song of D. melanogaster to act in this way, ipi might be expected to remain relatively constant throughout a courtship, to be relatively constant within a species, and to differ between species.

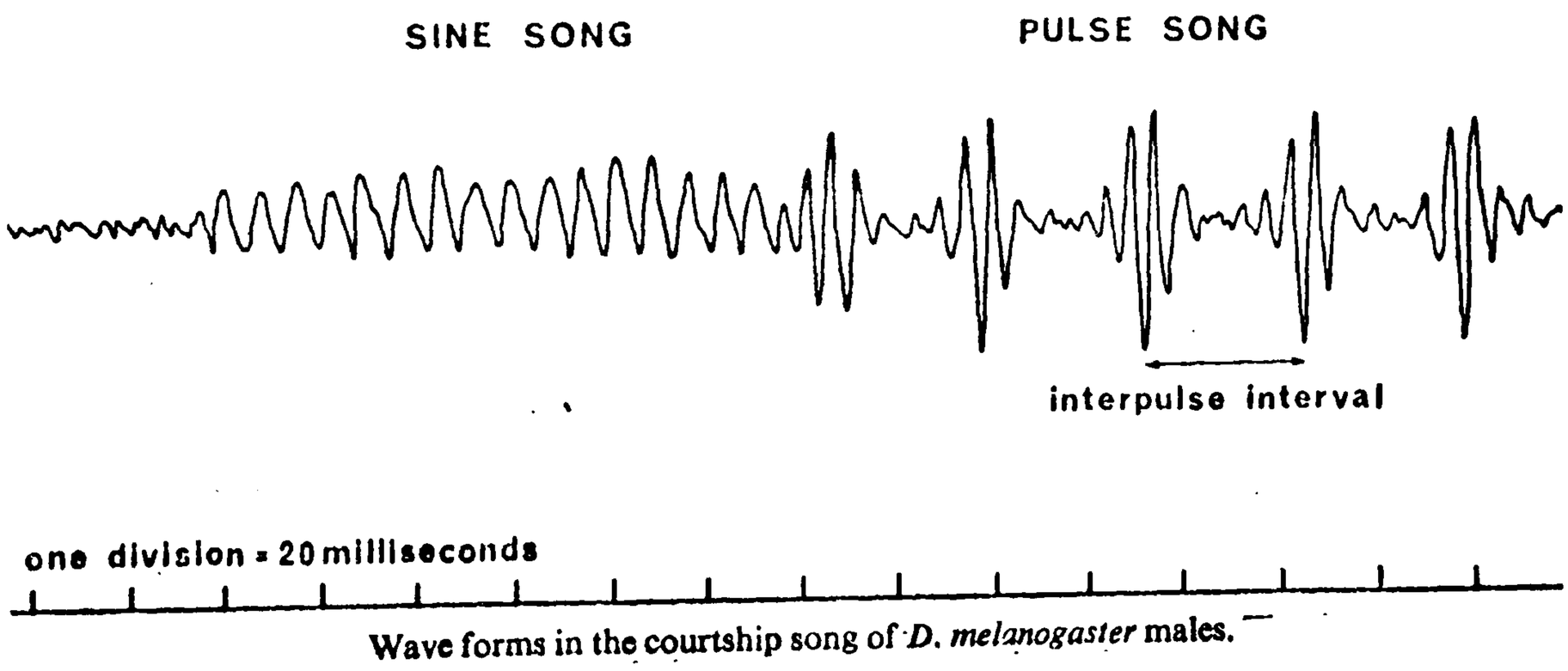
In this chapter the fine scale temporal patterning of ipi and of various other parameters of pulse song and sine song are investigated in courtships of Drosophila melanogaster.

Figure 17. Pulse song and sine song.



Oscilloscope traces of the courtship song of *Drosophila melanogaster*
Calibration signal : 100 Hz.

Figure 18. Parameters of the courtship songs.



Wave forms in the courtship song of *D. melanogaster* males.

Materials and methods

The courtship song of D. melanogaster has been studied in most detail in an attached-X chromosome strain (Schilcher, 1976c). Therefore, this strain has been used to examine the temporal patterning of courtship song. All males in this strain are wild-type; the females are mutant and do not survive long. This has apparently resulted in a fast mating speed for this strain (median time to copulation is 1 min; fewer than 2% of the courtships are longer than 5 min). Thus, the entire courtship can be recorded and analysed relatively easily.

The stock was mass cultured on standard Drosophila medium in bottles at 24+1 C and under a 12 hr light: 12 hr dark cycle. Flies were sexed under light ether anaesthetisation within 12 hr of eclosion, transferred in groups of 20 to 2.5 x 6 cm vials containing medium, and aged for 3 days. The following technique was used for recording courtship song. A male was introduced with a female into a 10 x 15 x 15 mm wire mesh cage with perspex top. The cage was placed on a ribbon microphone 1 mm above the ribbon, and the song was recorded, after appropriate amplification, with a Tandberg 3000X tape recorder. The recordings were then displayed on one beam of an oscilloscope with a calibration signal on the other beam. The oscilloscope traces were filmed at high speed and measurements were made from the film.

All experiments were conducted within 3 hr of the flies' dawn. The temperature during recording was controlled at 24.8 ± 0.1° C because of the strong temperature dependence of courtship song parameters (Shorey, 1962). In all cases, the entire courtship, from introduction of the flies until copulation, was recorded and analysed.

The durations of pulse song bouts, sine song bouts, and the

intervals between song bouts were found to be lognormally distributed (program A3.2 of Sokal and Rohlf [1969] was used in this analysis). Therefore, these measures were transformed to logarithms for these analyses. A song-free gap of more than 0.1 sec was defined as an interval between song bouts.

Temporal patterning of the various song parameters was detected by regression analyses. In all regressions the serial order of the bouts was used as a measure of time. Regression lines in different males were compared by analyses of covariance (Snedecor and Cochran, 1967, p. 432). All graphs in this thesis are idealized; they do not show any of the variability within or between courtships. Interpretation of the trends must rely on the tabulated analyses of variance.

Results

Table 31 presents the results of a comparison of the regression of pulse song bout durations on bout number during the courtships of 74 males. The analysis of covariance examines the fit of each male's data to a quadratic equation and indicates that the regressions were significantly curved. Males differed in both the slopes and the initial positions (elevations, y-intercepts, adjusted means) of their regression lines. Although the males differed significantly in their slopes, the overall population trend was for a decrease in pulse song bout durations during courtship (59 of 74 linear coefficients were negative, χ^2 = 26.2, d.f. = 1, P<< 0.001) with the quadratic term acting to slow the rate of decrease (60 of 74 quadratic coefficients were positive, χ^2 = 28.6, d.f. = 1, P<< 0.001). Closer examination shows that the last bout of pulse song before copulation did not conform to the trend. It was significantly longer than predicted from the regression equation (54 of 74 deviations from the trend line were

Table 31. Analysis of covariance of pulse song bout duration on bout number.

Term	D.f.	M.s.	F-ratio
Between positions	73	0.33	2.64
Combined slopes	2	0.55	4.39
Linear coeff.	1	0.75	5.99
Quadratic coeff.	1	1.06	8.47
Error	2048	0.13	
Divergence in slope	146	0.16	1.33
Error	1902	0.12	

Range of linear coefficients, -0.438 to 0.397 log sec.

Range of quadratic coefficients, -0.167 to 0.084 log sec.

Range in position, -1.567 to 0.525 log sec.

All F-ratios significant at $P < 0.025$

positive, χ^2 = 15.6, d.f. = 1, P< 0.001). This was not true for the 2nd, 3rd, or 4th last bouts of pulse song, which did fit the trend. Thus, there was a sudden increase in pulse song bout duration immediately before copulation.

Table 32 presents a similar analysis for the duration of sine song bouts. Although the males differed significantly in their initial bout durations they did not differ in their slopes. The pooled estimate of the slope was significantly different from zero; sine song bouts increased linearly in duration during courtship. Unlike pulse song bout durations, all sine song bout durations conformed to the trend.

Inter-pulse-interval

The analysis of covariance for the mean ipi per bout of pulse song (Table 33) indicates that the males did not differ in their slopes but did differ significantly in their initial ipis. The regression of mean ipi on bout number was significantly curved, and mean ipi per bout increased during courtship. However, examination of the mean ipi of the ultimate and penultimate bouts of pulse song prior to copulation reveals that these bouts did not conform to the general trend for increasing mean ipi. The last bouts showed a significant deviation from the trend line (58 negative and 16 positive deviations, χ^2 = 23.8, d.f. = 1, P<< 0.001) as did the second last bouts (39 of 74 deviations were negative, χ^2 = 7.78, d.f. = 1, P< 0.01). This was not true for the third, fourth or fifth last bouts of pulse song, and indicates that there is a sudden drop in mean ipi per bout before a successful copulation attempt. The overall mean ipi of the last pulse song bout (across all males) was 34.6 msec.

Table 32. Analysis of covariance of sine song bout durations on bout number.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	66	0.20	2.11	0.0050
Zero slope	1	0.82	8.59	0.0050
Error	726	0.10		
Equality of slopes	66	0.08	0.87	0.5000
Error	660	0.10		

Linear coefficient = 0.0044 log sec

Range in y-intercept, -1.536 to 0.148 log sec

Table 33. Analysis of covariance of mean ipi per bout of pulse song on bout number.

Term	D.f.	M.s.	F-ratio
Between positions	73	117.21	2.78+
Combined slopes	2	1423.08	33.79+
Linear coeff.	1	2232.66	53.02+
Quadratic coeff.	1	1111.93	26.40+
Divergence in slopes	146	30.42	0.72
Error	1902	42.11	

Mean linear coefficient, 0.1049 msec.

Mean quadratic coefficient, -0.000476 msec.

Range in position, 16.93 to 52.03 msec

+ $P < 0.001$

A more detailed analysis of the changes in ipi with time was undertaken by examining the first and last bouts of pulse song during courtship, and regressing each individual ipi within the bout on its serial order. Table 34 shows that during the first bout of pulse song, ipi increased linearly and that males differed in both their slopes of increase and their elevations. An analysis of covariance for the last bout of pulse song before copulation (Table 35) indicates that during this bout ipis increased linearly and that males differed in both the slopes and elevations of their regression lines.

A comparison of the slopes of the individual ipis within the first and last bouts of pulse song, with the slopes of the mean ipi per bout of pulse song throughout courtship indicates that individual ipis increased, on average, 3 to 5 times faster within both the first and last bouts than the mean ipi did during the entire courtships.

Since it is possible that the mean ipi of a bout of pulse song depends on the duration of the bout, it is necessary to examine the relationship between these two variables, especially as mean ipi increased during courtship and the duration of pulse song bouts decreased during courtship. Table 36 indicates that although mean ipi was related negatively to the duration of pulse song bouts, even when this relationship is taken into account, meanipi still increased during courtship.

Intervals between song bouts

The intervals between pulse song bouts and between sine song bouts decreased significantly in duration during courtship (Tables 37 and 38). Males were significantly different in the elevations but not in the slopes of these trends. Therefore, the frequency of pulse song

Table 35. Analysis of covariance of ipi during the last bout of pulse song.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	72	132.44	9.93	0.0010
Zero slope	1	349.23	26.19	0.0010
Error	1099	13.34		
Equality of slopes	72	39.60	3.44	0.0010
Error	1027	11.49		

Range of linear coefficient, -1.27 to 1.43 msec

Range in position, 24.94 to 47.05 msec.

Table 34. Analysis of covariance of ipi during the first bout of pulse song.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	75	91.14	5.59	0.0010
Zero slope	1	905.66	55.50	0.0010
Error	992	16.32		
Equality of slopes	75	55.87	4.27	0.0010
Error	917	13.08		

Range of linear slope, -0.689 to 5.00 msec.

Range in position, 17.11 to 37.52 msec.

Table 36. Analysis of covariance of mean ipi per bput of pulse song on bout number and bout duration.

Term	D.f.	M.S.	F-ratio
Between positions	73	108.7	3.18+
Combined slopes	2	1067.2	31.25+
Bout number coeff.	1	1658.0	48.56+
Bout duration coeff.	1	520.9	15.26+
Divergence in slope	146	38.9	1.14
Error	1726	34.2	

Mean bout number coefficient, 0.0346 msec.

Mean bout duration coefficient, -1.63 msec/sec of pulse song.

Range in position, 21.64 to 40.97 msec.

+ $P < 0.001$.

Table 37. Analysis of covariance of intervals between pulse song bouts on bout number.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	10	0.64	2.66	0.0250
Zero slope	1	1.24	5.17	0.0250
Error	883	0.24		
Equality of slopes	10	0.20	0.82	0.5000
Error	873	0.24		

Linear coefficient = -0.0009 log sec

Range in position, -0.857 to 0.206 log sec.

Table 38. Analysis of covariance of intervals between sine song bouts on bout number.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	18	0.85	2.58	0.0250
Zero slope	1	1.72	5.21	0.0250
Error	444	0.33		
Equality of slopes	18	0.32	0.98	0.5000
Error	426	0.33		

Mean quadratic coeff. = -0.0000634 log sec.

Range in position, -0.678 to 0.785 log sec.

and of sine song bouts increased during courtship.

Sequences

Examination of the sequence of pulse song and of sine song bouts reveals a significant tendency for the two types of song to alternate (Table 39a). Pulse song bouts that followed sine song bouts were significantly longer and had shorter mean ipis than those bouts that followed pulse song bouts (Table 39b).

Relationships to mating speed

Table 40 examines the relationship of initial sine song bout duration, of initial mean ipi per pulse song bout, of initial pulse song bout duration, and of pulse song bout slopes to mating speed (logarithmically transformed <Dow, 1976>). The multiple regression equation shows that only initial sine song bout duration and initial mean ipi per bout of pulse song were significantly related to mating speed. In both cases, the time to copulation was increased with an increase in the values of the variables (positive slopes).

Discussion

The temporal patterning of courtship song in Drosophila melanogaster is complex. All the behavioural variables measured in this chapter have significant trends with time even though the median time period involved is only about 1 min. This suggests caution when applying statistical methods that require stationarity to behavioural data, at least in D. melanogaster.

The variables can be grouped into two classes, those which differ significantly between males and those which do not. The members of the latter group are excellent candidates for use as

Table 39a. Sequential organisation of pulse song and sine song bouts. Only courtships with all expecteds > 5 are tabulated; 69 of 74 courtships showed more alternating couplets (SP and PS) than expected. P = pulse song bout, S = sine song bout.

Male	Observed frequency of the sequence				chi-square
no.	PP	PS	SP	SS	
3	30	17	18	4	2.29 NS
6	5	12	13	2	10.62
7	3	10	10	5	5.32
14	9	10	10	7	0.47 NS
17	3	8	9	3	5.24
19	15	16	17	0	13.16
21	43	43	43	3	24.95
25	4	11	11	1	11.41
28	14	18	18	3	9.33
29	32	29	29	1	17.78
39	85	28	28	5	1.35 NS
44	41	21	21	0	9.52
47	107	52	52	4	14.05
58	49	22	22	2	4.88
66	16	19	19	3	9.42
68	33	23	24	0	13.84
70	20	14	14	3	2.82 NS
74	16	2	2	9	14.50 opposite direction

Table 39b. Comparison of the duration of pulse song bouts and the mean ipi of pulse song bouts which follow sine song bouts or pulse song bouts (SP1 vs PP2)

	Mean bout duration		Mean ipi	
	P1 > P2	P2 > P1	P1 > P2	P2 > P1
No. of males	48	14	22	40
chi-square (1:1 expectation)	23.28		5.23	
Probability	0.001		0.025	

SP1 = P1 is a pulse song bout following a sine song bout S.

PP2 = P2 is a pulse song bout following a pulse song bout P.

Table 40. Multiple regression of time to copulation on courtship song parameters.

Variable	Coeff.	S.e.	Coeff.	S.e.
Mean ipi position	0.048	0.019	0.062	0.018
sine song bout duration position	0.343	0.258	0.506	0.228
Pulse song bout duration position	1.442	0.952	deleted	
linear coeff.	4.619	3.779	deleted	
quadratic coeff.	0.255	0.251	deleted	
Y-intercept	0.308	0.251	-0.293	0.646
F-ratio	2.49		8.08	
Probability	0.04		0.0007	

species identifiers (sexual isolation), if they are species-specific. Conversely, from the females's point of view, these variables would be useless for distinguishing between males of different fitnesses within the species (sexual selection). The first group could be used for this purpose if these variables were correlated with each male's fitness. However, although the first class of variables differs between males, they could still potentially be used as species recognition signals, if the variable ranges of different species did not overlap.

Females play an important role in determining the time of copulation, as indicated by the sudden change in pulse song bout durations and in mean ipi per bout immediately before copulation. Since these changes do not conform to their respective trends, this suggests that females 'choose' to mate after receiving specific stimulation and that pulse song bout durations and ipis are not summated (Ewing, 1964; Manning, 1966; Bennet-Clark, et al., 1973). Sine song bout durations may be summated by the female, as the ultimate sine song bouts do conform to their expected values.

We are faced with the question of which, if any, of these trends are adaptive. Since females appear to 'choose' to copulate immediately after longer than expected pulse song bouts with shorter than expected mean ipis (close to the overall species mean [Schilcher and Manning, 1975]), it might be concluded that trends for increasing mean ipi per bout and decreasing pulse song bout durations throughout courtship are maladaptive. Perhaps the experimental method of confining a pair of flies in a small cell until copulation is not normal: a reasonable assumption. If natural courtships are only a few seconds long (<10 sec?) and if during this time only one bout of courtship song is delivered then there would not be any trends in the song

parameters. Unfortunately, very little is known about the courtship behaviour of D. melanogaster in the natural environment. However, it is unlikely that every natural courtship with a virgin, sexually mature female results in copulation, as occurred in this experiment.

Further evidence for the speculation that natural courtships are very short is found in the comparison between trends of ipi within pulse song bouts and of mean ipi between pulse song bouts during the entire courtship. As the trends within bouts are three to five times greater than those across bouts, it follows that ipi increases during a bout of pulse song and declines to near its original level during the interval between bouts. In the natural environment the interval between pulse song bouts might be equal to the interval between courtships, and this could be long enough for the ipi mechanism to return to its starting point.

However, opposed to the suggestion that these trends are maladaptive is the finding that the initial mean ipi per bout is less than 34.6 msec (the mean ipi of the ultimate bout) and that it requires 6 bouts of pulse song for the mean ipi per bout to increase to this level. On average this requires 10 seconds of courtship. Furthermore, the increase in ipi with time is not confined to this strain. Wilson et al. (1976) also found increases in ipi in all (4) genotypes of D. melanogaster that they examined.

It is unknown whether female D. melanogaster can detect the fine scale differences involved in the trends of ipi, particularly the within-pulse-song-bout trends. Males have very poor discriminatory powers (Schilcher, 1976a), although they are most sensitive in the range 30 to 40 msec, which is included in the range of ipis produced during average length courtships. Males, of course, are not expected to be as critical as females (Trivers, 1972).

Zaretsky (1972) has shown that female crickets, Scapsipedus marginatus, will respond phonotactically only to the correct pulse interval pattern of the male calling song. The pulse interval in this species is bimodally distributed (33 and 64 msec) with the long intervals and the short ones alternating. Zaretsky (1972) found that females would not respond to calling songs with pure 33 msec intervals, pure 64 msec intervals or pure 49 msec intervals (mean of 33 and 64) but would respond to alternating 33 and 64 msec pulse intervals. Other parameters of the calling song, such as the dominant frequency and chirp interval, could be drastically changed without affecting the attractiveness of the male calling song to the female. Thus, females may show extreme sensitivity to some parameters of song and insensitivity to other parameters. Female D. melanogaster are known to be sensitive to ipi (Table 40 and Schilcher, 1976a,b). We do not know if they are sensitive to trends in mean ipi per bout and whether the trends are species-specific.

Evidence suggests that the trends of sine song bout durations, of sine song intervals and of pulse song intervals may be adaptive. Sine song has been shown to stimulate females to copulate (Schilcher, 1976b) and therefore, increasing both the duration and frequency of sine song bouts would appear to be adaptive. Pulse song bouts have also been shown to stimulate females to copulate, but this stimulation is not capable of being stored (Schilcher, 1976b). Thus an increase in the frequency of pulse song bouts would also appear to be adaptive.

Chapter 6. Sequential analysis

Analysis of behavioural sequences in D. melanogaster may provide further information about the organisation of male courtship behaviour and about behavioural interaction between the sexes. Connolly and Cook (1973) examined the causes of female rejection behaviour and the effects of the female's behaviour on the male. They stated "No changes in the males' behaviour were observed following fending by the females" and concluded "the data do not give strong support to the view that flicking and kicking have significant effects on the behaviour of the male" and "the major effect of extrusion ... is to prevent the male copulating"

Manning (1959) looked at the sequential organization of courtship behaviour in male D. melanogaster and D. simulans. He found that the most frequent transition series was, ORI -> VIB -> LIC -> ATT.

Results

Before examining transitions in the courtship behaviour of D. melanogaster the assumptions upon which such analyses are based must be tested. These include stationarity of the data (constant probability of occurrence of behavioural acts over time) and, if the data from many courtships are pooled, homogeneity of the data sets. The findings of chapter 4 were that the data were not stationary. The chi-square analysis of sequence contingency tables when there are trends in the probabilities of occurrence of behavioural elements becomes biased. Appendix 3 clearly shows that chi-square is inflated when frequencies are pooled either over time or between individuals when such data sets are different. Fortunately, the effect appears small for large differences in frequencies. However, it is not difficult to devise methods to account for such trends, etc. (see

Appendix 3 for the methods).

Male behaviour

The first aspect of the males's courtship behaviour that must be tested for heterogeneity is the frequency of each behavioural pattern per courtship. The heterogeneity chi-square for this test was 392.5 with df = 496 and was not significant. Therefore, the data can be assumed to be homogeneous. The typical courtship consisted of 36.5% orientation, 32.9% vibration, 12.0% lick, 8.5% attempted copulation and 10.1% non-courtship (percentages of the total number of events per courtship).

A similar test for heterogeneity can be applied to compare the dyad (couplet) transition matrices of each male and indicates that these data were also homogeneous (χ^2 = 1023.2, df = 1220, NS). Therefore, the data from all courtships may be pooled (Table 41) and tested to determine whether dyad frequencies were random. Expecteds for this analysis were calculated by Goodman's method (1968). (It should be mentioned that Morgan (1977) has compared iterative methods for obtaining maximum likelihood estimates in tables with missing diagonals.) The results of this test (χ^2 = 3622.0, df = 11, P<<0.001) indicate that the probability of occurrence of a behavioural act depended upon at least the identity of the preceding act.

Lewontin and Felsenstein (1965) have shown that the chi-square test is valid as long as all expecteds are greater than 0.5. However, some of the individual transition tables for the males have expecteds that are smaller than this. Although this should tend to inflate the chi-square and produce spurious significant results rather than non-significant results as found in the analysis, it is worthwhile to repeat the heterogeneity test on the individual transition tables

using the method advocated by Everitt (1977). It may prove useful to mention here that Baker (1977) has provided a computer algorithm for calculating exact probability distributions for two-dimensional contingency tables. This generalises the Fisher test for 2 X 2 tables to R X C tables.

For Everitt's method the expecteds are calculated separately for each male's transition matrix and then summed and compared with the summed observeds by a chi-square test. This analysis for the dyad transitions gives χ^2 = 2757.1, df = 11, P<< 0.001 and agrees with the previous method, although the chi-square is somewhat smaller, in finding that the behavioural organisation of the males is not random. The significance of the various sources of variability is summarised in Table 40.5. As shown in this table the reduction in the overall chi-square due to fitting regressions to each courtship did not significantly change the conclusion that the courtship acts of the male are associated sequentially.

Since the overall χ^2 test was significant the individual cells of the table may be examined to detect which dyads contribute significantly to the non-randomness detected by the overall test. Many methods have been used to do this in the past; see Appendix 4 which presents a further method. Using this type of analysis all the cells in Table 41 were found to be significantly different from their expecteds.

It is now possible to examine the next order sequence. Although correction for non-stationarity did not alter the significance of the dyad transition table (Appendix 3), analysis of the triads reveals that the triad frequencies did not differ significantly from their expecteds when the non-stationarity was taken into account.

Figure 19 presents the most frequent dyad transitions. Other

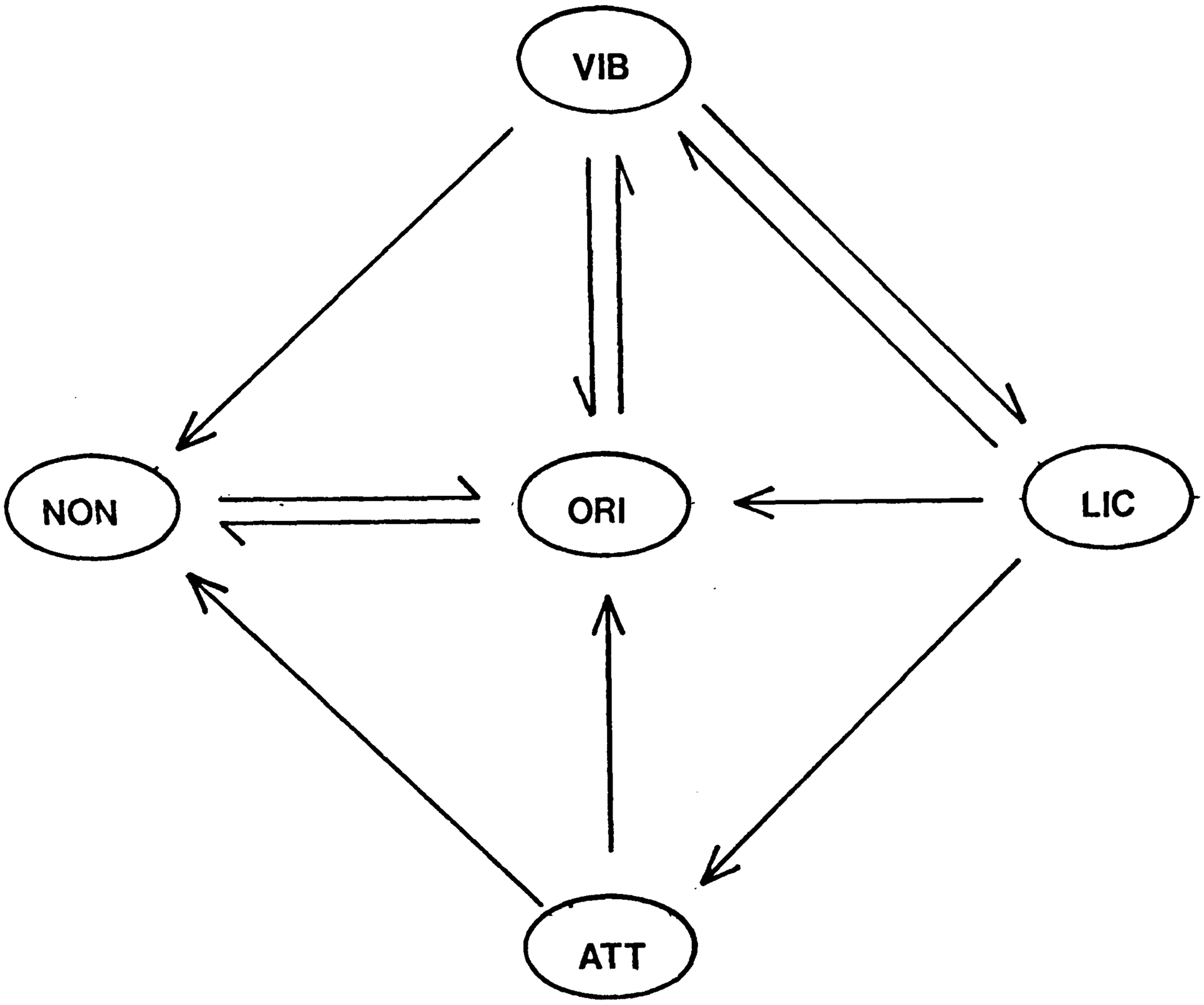
Table 40.5. Chi-square analysis of sequences.

	Chi-square
Overall	5245.80
Reduction for zero diagonals	1623.84
Reduction for courtship heterogeneity	316.67
Reduction for trends	486.07
Remainder	2819.22

Table 41. Sequential linkage of courtship elements in wild-type males. Preceding acts are represented by rows;following acts by columns.

	ORI	VIB	LIC	ATT	NON
ORI		1073	2	2	267
VIB	670		436	33	70
LIC	54	97		275	15
ATT	147	18	2		21
NON	352	18	2	1	

Figure 19. Frequent transitions between male courtship elements.



transitions that apparently occur (e.g ORI -> ATT, Table 41) are considered to be artefacts due to the short durations of the behaviour patterns, and my reaction time. On some occasions short intervening acts will have been missed and on others acts may have been incorrectly overlapped. This may lead to gaps or overlaps in the behavioural record. These were rectified automatically by the computer in favour of the most recent behaviour. The reality of the unusual transitions in Table 41 can only be verified by single frame analysis of filmed courtships.

Female behaviour

No attempt was made to fit expecteds to the female dyads or to calculate a chi-square since the female behavioural acts are not mutually exclusive. The expecteds would therefore have to be based on their durations as well as their frequencies. Sequence analysis of such behaviour is the same as asking what is the probability of interrupting a behaviour event, and becomes complicated if behavioural patterns are allowed to overlap more than one other pattern, as female behaviour can.

Nevertheless the `dyad` transition frequencies (Table 42) may be examined to some extent because run and stand are mutually exclusive acts that occupy the entire courtship. Since the total duration of runs was three times that of stands any behaviour pattern that occurred more frequently after stands than after runs could be considered to be associated with standing. This was true for fending, elevating, extruding and preening. The other association of interest is between elevating and kicking. The total duration of elevations was less than that of both extrusion and preenings yet 80 kicks followed elevations and only 12 followed extrusions and preenings

Table 42. Sequential linkage of female courtship acts. Details as for table 41.

	RUN	STA	FEN	ELE	FLI	KIC	EXT	PRE	SPR
RUN		774	9	80	11	20	7	2	4
STA	508		40	112	4	13	60	86	0
FEN	24	11	3	5	1	5	6	12	0
ELE	68	55	4	23	6	80	9	3	0
FLI	3	10	0	6	3	1	0	1	2
KIC	48	36	2	24	3	8	1	10	0
EXT	56	6	1	15	0	5	4	1	0
PRE	82	4	10	11	0	2	2	15	1
SPR	0	4	0	1	0	0	0	0	0

combined.

Male-female behavioural interaction

Table 43 shows the dyad frequencies summed across all courtships. The behaviour of the male and the behaviour of the female of each pair was merged into a combined sequence on the basis of the starting times of the behaviour patterns (Fig. 20). The overall sequence of behaviour was based on which behaviour pattern started next, regardless of the sex to which it belonged.

Expecteds were calculated for the male-female transitions using the total durations of the male patterns and are shown for selected transitions in Table 44. It would appear that fends were significantly positively associated with vibration. Elevations were positively associated with licks and attempted copulations. Kicks were positively associated with orientations, licks and attempted copulations. Extrusions were positively associated with vibrations, licks and attempted copulations.

Examination of the female-male transitions suggests that licks and attempted copulations were associated with standing and that both these behaviour patterns were frequently preceded by elevations. Few licks and attempted copulations followed extrusions.

Discussion

That the frequencies of the male behavioural patterns are homogeneous across courtships suggests that there is only one factor or underlying variable that determines the proportions of the various courtship events. It also shows that all males court similarly and that the variability present in the frequencies is due to the effects of random sampling (finite sample sizes). Both these points are

Table 43. Sequential linkage of male and female acts combined.

Details as in table 41.

	ORI	VIB	LIC	ATT	NON	RUN	STA	FEN	ELE	FLI	KIC	EXT	PRE	SPR
ORI		707	1	1	184	150	150	14	45	8	48	8	27	1
VIB	460		190	21	53	87	263	23	57	10	11	20	11	4
LIC	46	79		207	12	8	44	2	31	0	5	7	0	0
ATT	68	7	1		10	28	25	0	48	0	14	12	1	0
NON	208	7	2	0		37	97	2	11	0	4	2	3	0
RUN	272	196	40	4	63		286	6	29	2	10	3	1	2
STA	136	86	130	36	26	315		188	38	3	4	26	68	0
FEN	16	11	8	1	4	16	2	1	1	0	1	3	5	0
ELE	62	27	38	25	11	40	16	1	2	2	34	6	0	0
FLI	6	9	4	2	2	3	0	0	0	2	0	0	0	0
KIC	29	27	7	6	1	35	10	1	7	1	3	0	7	0
EXT	17	9	13	8	4	26	2	0	7	0	0	2	1	0
PRE	23	44	4	0	3	44	4	1	1	0	0	0	6	0
SPR	1	1	4	0	0	0	1	0	0	0	0	0	0	0

Figure 20. Method used to merge the male and female behavioural sequences.

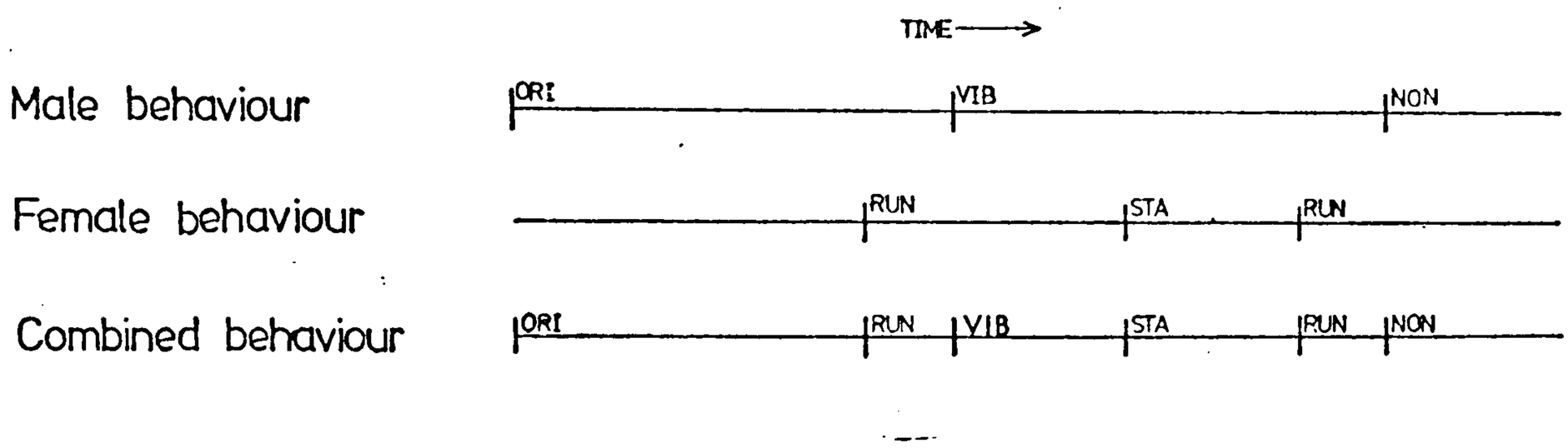


Table 44.

Selected male-female transitions.

Transition	Expected	Observed
VIB -> FEN	11.3	23
LIC -> ELE	3.1	31
ATT -> ELE	6.8	48
ORI -> KIC	30.0	48
LIC -> KIC	1.3	5
ATT -> KIC	3.1	14
VIB -> EXT	13.5	20
LIC -> EXT	0.8	7
ATT -> EXT	1.9	12
ORI -> FEN	15	14
VIB -> ELE	53	57
VIB -> KIC	22.6	11
ORI -> ELE	70	45

All values significant at least at the 5% level.

contradicted by the findings of the previous chapter. However, chi-square analyses have low power in tests of specific hypotheses (Siegel, 1956) and the significant trends found in the frequencies of the behaviour patterns may not be large enough to be detected by such tests. Moreover, sequence analysis does not involve any measure of time other than the order in which the behavioural events occur. Analyses of covariance of the probability of occurrence of the male behaviour patterns against serial order indicates that the males are homogeneous (data not shown).

Sequence analysis of the male's courtship behaviour indicates that various acts are tightly linked. The high frequency of the sequence ORI -> VIB -> LIC -> ATT was confirmed (Manning, 1959). This should not surprise us; vibration is superimposed on orientation, and licks and attempted copulations are superimposed on vibration. As successful copulation is the male's aim when he courts a female, it is entirely reasonable that a copulation attempt should occur after he has displayed his entire courtship repertoire. This sequence would be unexpected only if bouts of vibration frequently started simultaneously with bouts of orientation and licks frequently occurred at the very beginning of vibration bouts.

It is interesting to note that attempted copulations are often followed by orientation or non-courtship. If these two behavioural elements represent low levels of sexual excitation (Bastock and Manning, 1955) then perhaps attempted copulation can be assumed to be similar to a consummatory act (Tinbergen, 1951). Connolly and Cook (1973) indicated that kicking occurred only when the female was standing. Although I subjectively agree with their finding, the sequence analysis suggested that kicks occurred as frequently while the female was running as when she was standing. This may be partly

an artefact due to my reaction time. Subjective assessment of the female's behaviour suggests that the female may be moving slowly with the male close behind her, she then stops, the male licks, she kicks and moves on. The duration of the stationary bout is so short that it would be difficult to respond to it by pressing an event recorder button. Such questions are best answered by single frame analysis; Dr. H. Bennet-Clark (personal communication) has estimated that kicks may have durations shorter than 0.05 sec. Crossley (1963) also found that kicks occurred while the female was running.

Connolly and Cook (1973) found that fending occurred when the male was close to the female and about to vibrate. The results of the male-female interaction analysis confirm their suggestion. No attempt has been made to analyse the behavioural events leading to flicks and spreads due to their low frequency. However, elevations, which Connolly and Cook (1973) found to be infrequent, were analysed and found to be significantly associated with both licks and attempted copulations in the male, and with kicks in the female. Kicks were also associated with orientation bouts and this may be because both licks and attempted copulations have short durations and are often immediately followed by orientation. Extrusions were infrequently followed by attempted copulations, which does not confirm the findings of Connolly and Cook (1973) but does support those of Bastock and Manning (1955). Extrusions were often given in response to licks and attempted copulations.

Chapter 7. Male-female behavioural interaction

To analyse the effect female behaviour has on the sequential organization of the male's courtship behaviour a method similar to that of Connolly and Cook (1973) can be used. However, I have made a number of changes to their method and therefore I shall describe the procedure in detail.

First, only occasions when one female act intervened between two consecutive male courtship behaviour patterns are used (Fig. 21). This reduces the complexity of possible interactions which might occur if the female performed two behaviour patterns between two male courtship acts. For example,

1) only the first female act has an effect

2) only the second female act has an effect

3) the two acts sum in effect

4) the two acts cancel in effect

5) the two acts interact non-additively in effect.

Secondly, a control or null set of transitions is required. This was provided by all transitions during which the female's behaviour was constant.

Figure 22 shows the relative proportions of the five male behavioural categories before and after the control transition. These are not equal since each dyad is totally independent and in fact the chi-square was highly significant. The male's behaviour was not sequentially organized at random. This must be borne in mind when the effect of the female's behaviour is examined.

Figure 22 also shows the relative frequencies of the males' behavioural elements before and after single interruptions by female behaviour patterns. For six of the nine female behaviour patterns the male's next behavioural act was significantly different from his

Figure 21. Definition of an interruption of the male's behaviour by a transition in the female's behaviour. Only situations like those in (a) were used; those like that in (b) were not used.

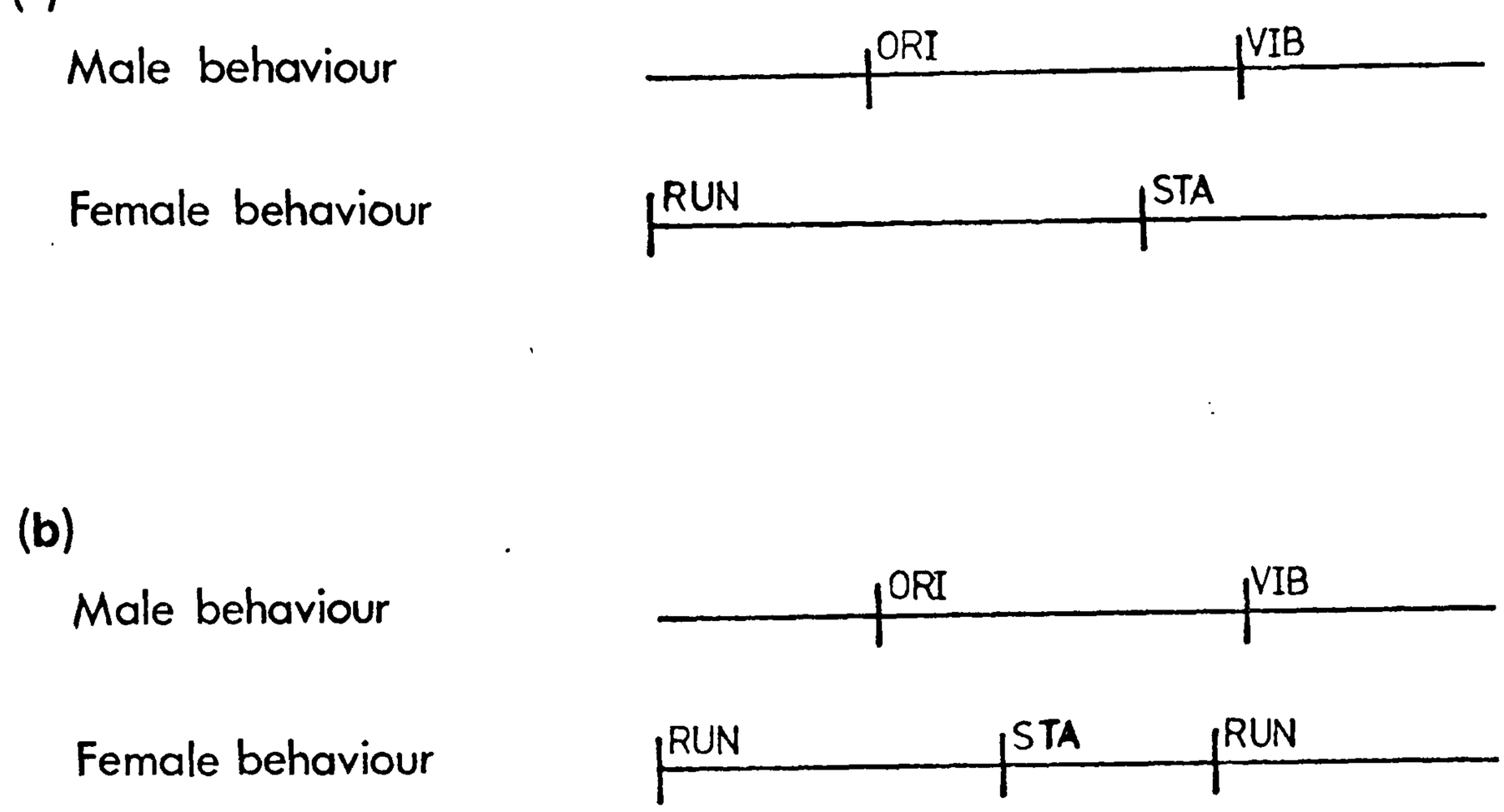
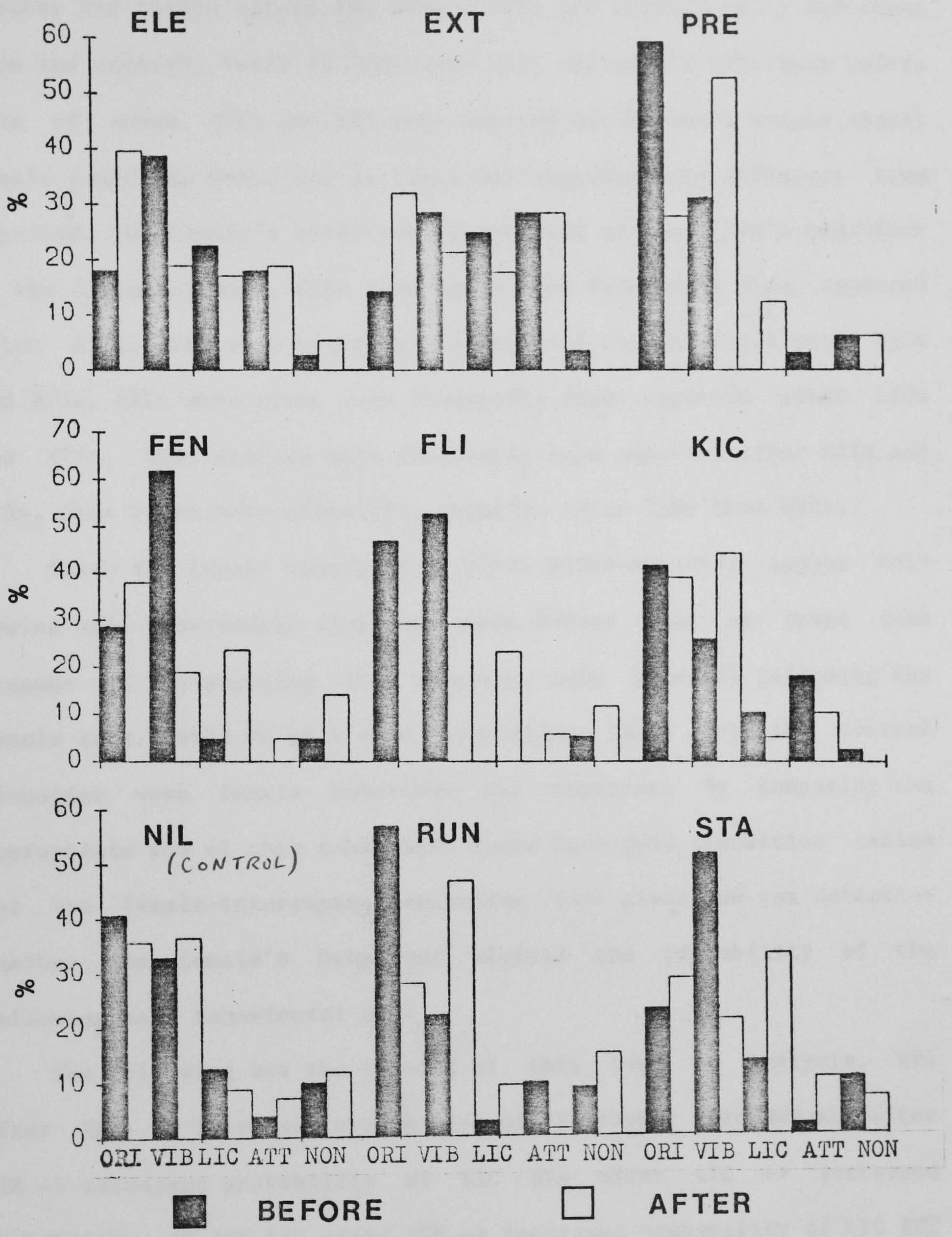


Figure 22. Frequency of male behaviour patterns before and after female behaviour patterns.



previous behavioural act. This is only to be expected from the result of the analysis of the control category. Therefore we must test whether the totals before the female acts are significantly different from the control. Table 45 indicates that the male's behaviour before five of seven (FLI and SPR were omitted due to small sample sizes) female courtship behaviour patterns was significantly different from expected. The female's behaviour was related to the males's behaviour in the following ways, KICs were given more frequently than expected after ATTs, ELEs were given more frequently than expected after LICs and ATTs, EXTs were given more frequently than expected after LICs and ATTs, RUNs started more frequently than expected after ORIs and ATTs, STAs began more often than expected after VIBs than ORIs.

Since the female behaviour patterns differentially appear following male behavioural elements, these biases must be taken into account in determining the expected male elements following the female acts. Table 46 is a dyad transition table for the control situation when female behaviour was constant. By comparing the appropriate row of this table with those from dyad transition tables for the female-interrupted sequences (not given) we can determine whether the female's behaviour altered the probability of the following male behavioural act.

The following are the results of this type of analysis, KIC after ORI -> increased probability of VIB rather then NON ELE after VIB -> increased probability of LIC STA after LIC -> increased probability of ATT EXT after VIB -> increased probability of LIC EXT after LIC -> increased probability of ATT. It is possible that when a KIC follows an ORI the male is closer to the female than when ORI is not followed by KIC and that on those occasions there is a higher probability that the male will continue to court solely due to his

Table 45. Male behaviour patterns preceding female acts.

	NIL	RUN	STA	FEN	FLI	KIC	ELE	EXT	PRE	SPR
ORI	893	128	6	6	8	16	20	4	19	1
VIB	721	50	33	13	9	0	43	8	10	4
LIC	276	6	73	1	0	4	25	7	0	0
ATT	86	22	163	0	0	7	20	8	1	0
NON	217	20	33	1	0	1	3	1	2	0
Chi-square		52.8	52.8	8.4		21.5	74.2	49.1	7.2	
Prob.		0.005	0.005	NS		0.005	0.005	0.005	NS	

Table 46. Male transitions during constant female behaviour.

	ORI	VIB	LIC	ATT	NON
ORI		707	1	1	184
VIB	460		190	18	53
LIC	46	79		139	12
ATT	68	7	1		10
NON	208	7	2	0	

Female

					interruption	Chi-square	Df
ORI		16	0	0	0	KIC	4.15	1
VIB	20		18	5	0	ELE	4.93	1
VIB	2		5	1	0	EXT	5.28	1
LIC	1	9		32	1	STA	10.38	3
LIC	0	0		7	0	EXT	6.74	1

All chi-square values significant at $P < 0.05$. Degrees of freedom depend on the exact hypothesis being tested.

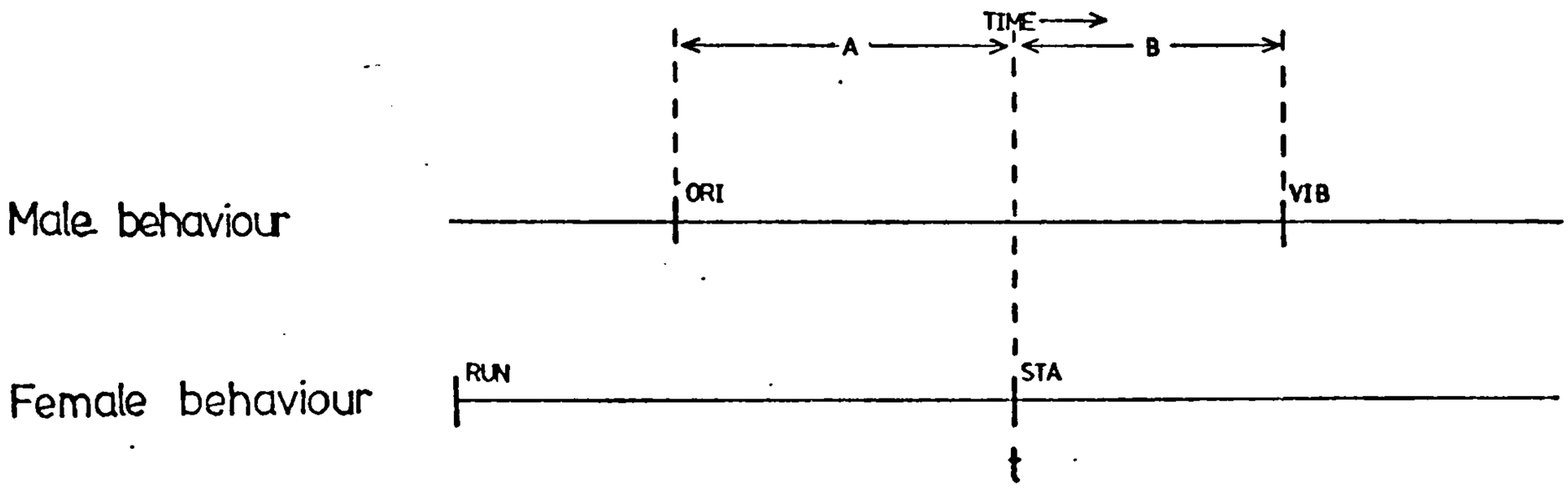
closeness to the female and not as an effect of having been kicked. Also ELE after VIB may be a response of the female to being grabbed by the male's sexcombs prior to a LIC, and an increase in the probability of a LIC occurring under these conditions may not be an effect of the female's having elevated. The results of the analysis for EXT are similar to those for Connolly and Cook (1973). There is no evidence from the sequential analysis for an inhibitory effect of the female behaviour patterns: KIC, ELE and EXT.

Durations

A second method for examining the interaction between male and female behaviour is to analyze the durations of behaviour patterns in one sex that are interrupted by the start of the behaviour of the other sex. For example if the male is orientated and the female begins standing how does this transition alter the remaining duration of the male's orientation bout? This type of question is answered in the following way. All bouts of orientation in a courtship are classified into a number of exclusive categories, a) not interrupted by any female behaviour i.e. the female's behaviour remains the same during the entire bout of orientation b) interrupted only once by the female starting to stand c) to i) interrupted only once by the female starting to run, preen, extrude, elevate, kick, flick, and fend j) interrupted more than once by any combination of female behaviour.

A random hypothesis would suggest that the longer the bout of orientation the higher the probability that it will be interrupted by a female behaviour pattern. This must be taken into account in the analysis and can be done in the following way (see Fig. 23) At time t the female starts to stand and the male has been orientated for a seconds. He continues to orientate for a further b seconds after the

Figure 23. Method used to determine the duration of a bout of behaviour in one sex following a transition in behaviour of the other sex.



female transition in behaviour. If we examine all the uninterrupted bouts of orientation that are at least a units in duration and subtract a units from each such bout and calculate their mean, then we have an estimate for the expected amount of time a male will remain orientated after already being orientated for a units, when the female's behaviour has remained constant. The difference between our mean expected and b is an estimate of the effect of the female's transition to standing on the duration of orientation. This can be done for every bout of orientation that was interrupted by standing and the differences so produced examined to determine whether their mean is significantly different from zero.

Table 47 examines whether the duration of orientation bouts was affected by being interrupted by female behaviour. Whenever the female started to stand or run, the following amount of orientation was less than would be expected if the females's behaviour had remained constant. Interruption by all other female behaviour patterns did not have significant effects on the duration of orientation bouts. Vibration bouts (Table 48) were also shorter than expected when interrupted by a transition from standing to running or vice versa. However, they were also decreased by transitions to preening and elevating. The duration of non-courtship bouts was decreased by transitions in the female from running to standing (Table 49).

Table 50 examines the effect that a transition in male behaviour has on the duration of standing bouts in the female. A transition to orientation was followed by a shorter than expected amount of standing and a transition to non-courtship was followed by a longer than expected amount of standing. Transitions to vibration and orientation were followed by less than expected amounts of running in the female (Table 51) and neither transition had an effect on the

Table 47. Duration of female-interrupted orientation bouts.

Number of bouts	Female behaviour							
with durations	STA	RUN	PRE	EXT	ELE	KIC	FLI	FEN
> Expected	10	48	8	1	12	12	3	1
< Expected	52	73	9	1	9	5	5	5

Table 48. Duration of vibration bouts interrupted by female behaviour.

Numbers of bouts	Female behaviour							
with durations	STA	RUN	PRE	EXT	ELE	KIC	FLI	FEN
> Expected	21	14	2	4	4	1	4	2
< Expected	102	32	7	3	27	6	4	8

Table 49. Duration of non-courtship bouts interrupted by female behaviour.

Number of bouts	Female behaviour					
with durations	STA	RUN	EXT	ELE	KIC	FEN
> Expected	3	8	0	0	0	1
< Expected	15	10	1	3	3	0

Table 50. Duration of standing bouts interrupted by male behaviour.

Number of bouts	Male behaviour				
with durations	ORI	VIB	LIC	ATT	NON
> Expected	15	5	11	1	0
< Expected	35	12	3	2	1

Table 51. Duration of running bouts interrupted by male behaviour.

Number of bouts	Male behaviour				
with durations	ORI	VIB	LIC	ATT	NON
> Expected	7	9	0	0	1
< Expected	25	54	1	0	2

amount of preening that was followed by it (Table 52).

Discussion

It is clear that transitions in either sex do affect the durations of behaviour patterns in the other sex. The interpretation of these effects is not unequivocal. In the majority of cases transitions during a behaviour in one sex lead to shorter than expected amounts of that behaviour after the transition. However, these can be explained by a) assuming that the transition has inhibited the animal and thus a stimulatory behaviour pattern has been shortened, or b) assuming that the transition has excited the animal and thus a stimulatory behaviour pattern has been shortened so that the animal can display a more stimulatory behaviour pattern sooner. For example, orientation bouts are reduced in duration by a transition from running to standing. This can be interpreted as being a stimulatory effect if the male goes on to vibrate but as an inhibitory effect if the male stops courting. Similar, ambiguous, explanations can be provided for all the effects shown in the tables. In the previous section on the sequential interaction the conclusion was reached that the female behaviour patterns did not inhibit the male. Equally they did not stimulate the male. Apparently, transitions in behaviour in one sex have a general decremental effect on the bout durations of the other sex. This was not true for transitions to non-courtship in the males which caused the female to stand for longer than expected. Thus, courtship by the male stimulates the female to run.

Probability of occurrence

A third method to determine whether there is male-female

Table 52. Duration of preening bouts interrupted by male behaviour.

Number of bouts	Male behaviour	
with durations	ORI	VIB
> Expected	2	6
< Expected	2	5

interaction during courtship is to examine the probability of occurrence of male behavioural acts during specific female behaviour patterns and vice versa. If there is no interaction between the sexes the probability of occurrence will remain constant, whereas the presence of interaction will appear as a trend in the probability of occurrence (either decreasing or increasing).

Probability of female behaviour patterns occurring during male ones

As shown in Table 53 and Fig. 24 the probability that the female will begin a bout of standing declined during orientation. The decline followed a quadratic trend and all females began with the same y-intercepts and followed the same trend. A similar situation was found when the probability that a female started a bout of running during orientation was examined (Table 54, Fig. 25). All females followed the same quadratic decline in probability and they all began with the same y-intercept. A quadratic decline was also found for the probability of a bout of elevating starting (Table 55, Fig. 26) and for the probability of the female kicking during orientation bouts (Table 56, Fig. 27). Again, for both elements, the females began with the same y-intercepts and followed the same trend. No significant trends were found for the probability of extrusion or preening during bouts of orientation (data not shown).

Vibration

The probability that bouts of standing will begin declined linearly with time during bouts of vibration (Table 57, Fig. 28). Females began with the same y-intercept and followed the same trend. This was also true for the probability of a bout of running starting during vibration (Table 58, Fig. 29). No significant trends were

Table 53. Analysis of covariance of the probability of the female beginning to stand during ORI bouts.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.62	4.10	0.0250
Linear coefficient	1	0.75	4.92	0.0500
Quadratic coefficient	1	0.16	1.04	NS
Divergence in slopes	124	0.11	0.73	NS
Between positions	62	0.09	0.56	NS
Error	584	0.15		
Linear coefficient =	-0.0408			
Quadratic coefficient =	0.0020			
Y-intercept =	0.2439			

Figure 24. Regression of the probability (x100) of occurrence of STA (A) during bouts of ORI (T).

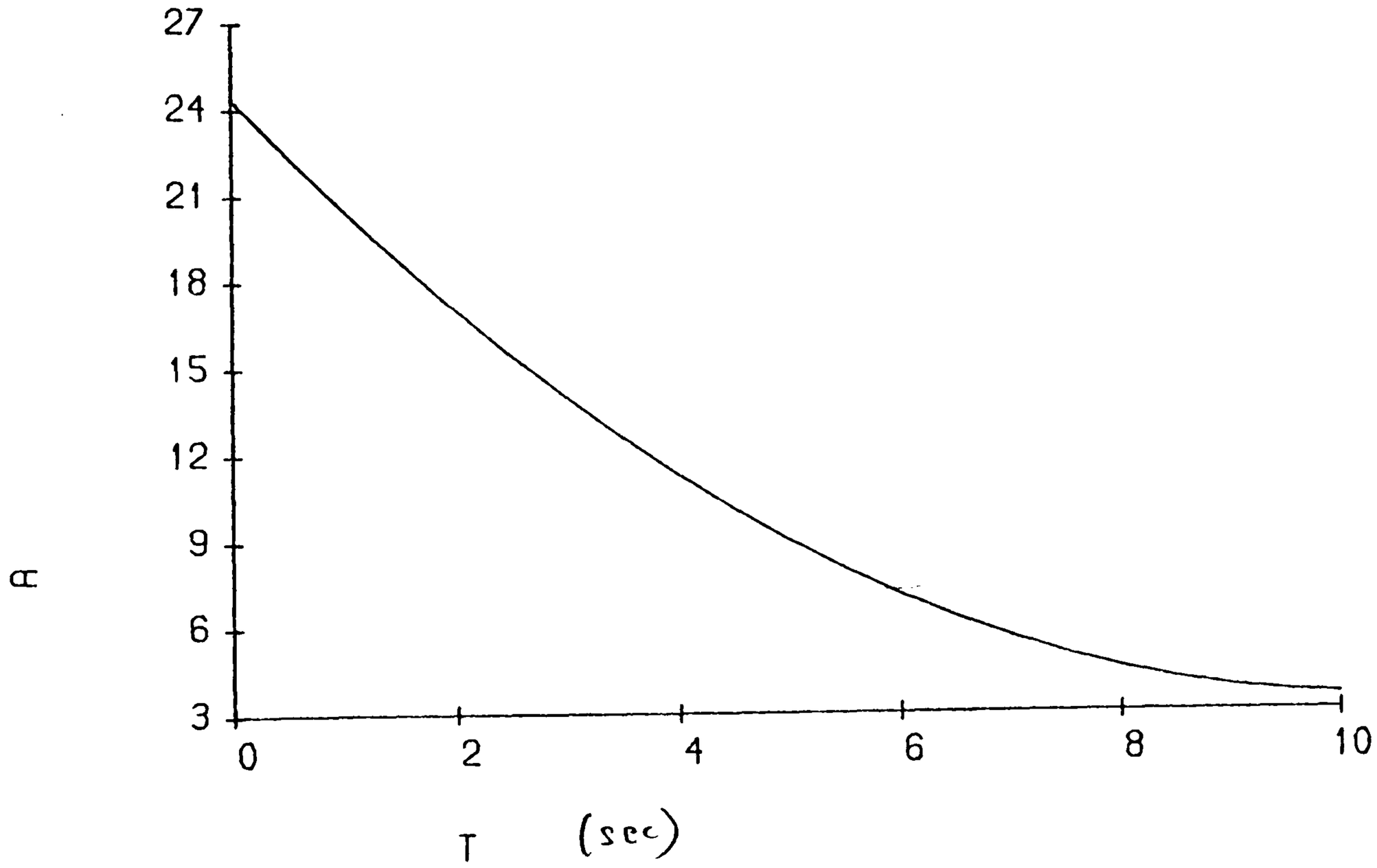


Table 54. Analysis of covariance of the probability of the female starting to RUN during ORI bouts.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	2.46	16.34	0.0010
Linear coefficient	1	3.56	23.68	0.0010
Quadratic coefficient	1	1.12	7.43	0.0100
Divergence in slopes	126	0.16	1.07	NS
Between positions	63	0.11	0.73	NS
Error	556	0.15		
Linear coefficient =	-0.0987			
Quadratic coefficient =	0.0075			
Y-intercept =	0.2849			

Figure 25. Regression of the probability (x100) of occurrence of RUN (B) during bouts of ORI (T).

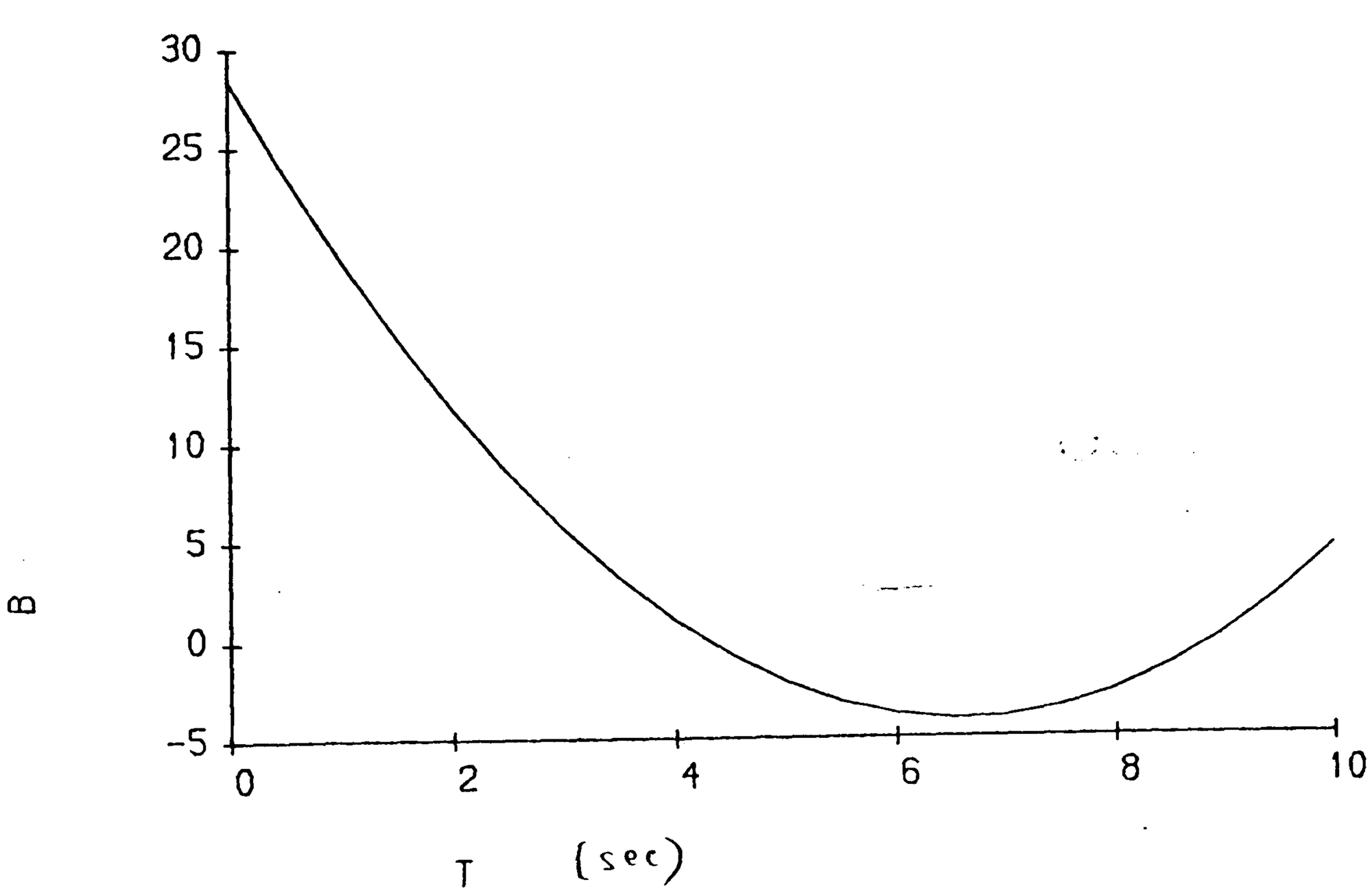


Table 55. Analysis of covariance of the probability of the female starting to elevate during ORI.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.94	10.24	0.0010
Linear coefficient	1	1.50	16.40	0.0010
Quadratic coefficient	1	0.70	7.66	0.0100
Divergence in slopes	58	0.09	0.96	NS
Between positions	29	0.06	0.68	NS
Error	336	0.09		
Linear coefficient =	-0.1074			
Quadratic coefficient =	0.0124			
Y-intercept =	0.2825			

Figure 26. Regression of the probability of occurrence of ELEs (C) during ORI bouts (T).

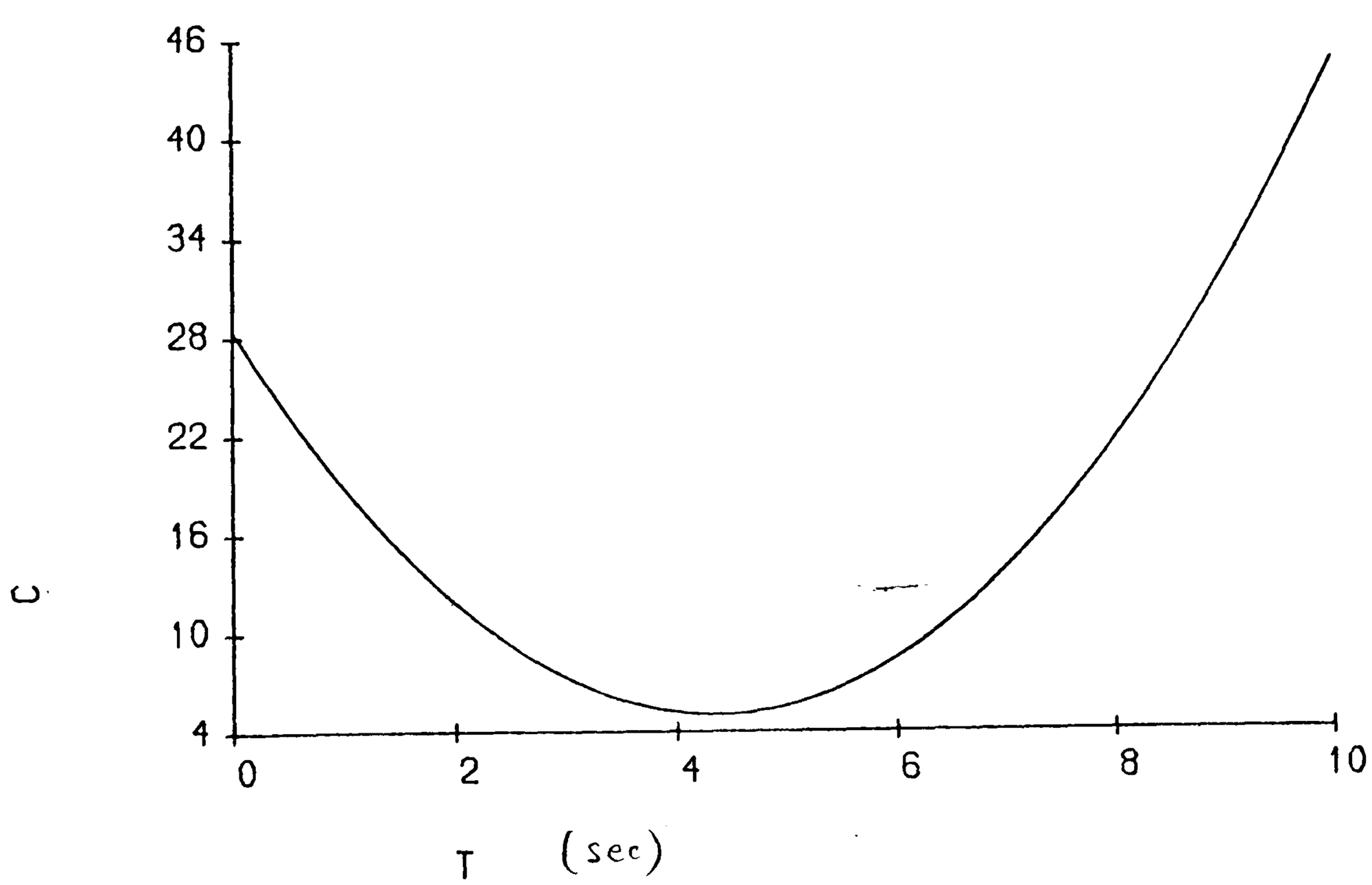


Table 56. Analysis of covariance of the probabilty of the female kicking during ORI.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	1.50	12.25	0.0010
Linear coefficient	1	2.62	21.41	0.0010
Quadratic coefficient	1	1.41	11.56	0.0010
Divergence in slopes	44	0.08	0.62	NS
Between positions	22	0.08	0.65	NS
Error	247	0.12		
Linear coefficient =	-0.1668			
Quadratic coefficient =	0.0200			
Y-intercept =	0.3180			

Figure 27. Regression of the probability of the occurrence of KICs (D) during bouts of ORI (T).

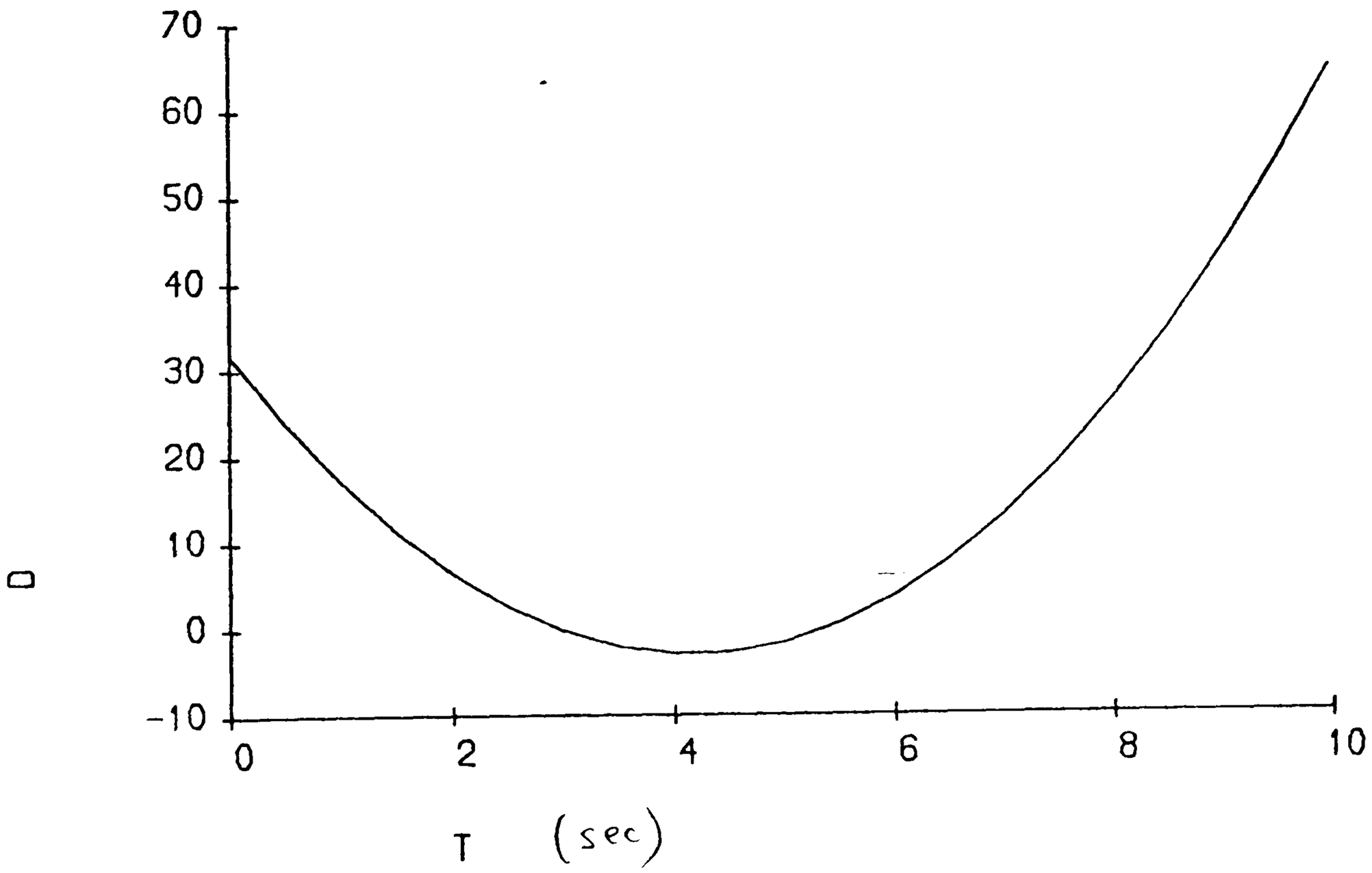
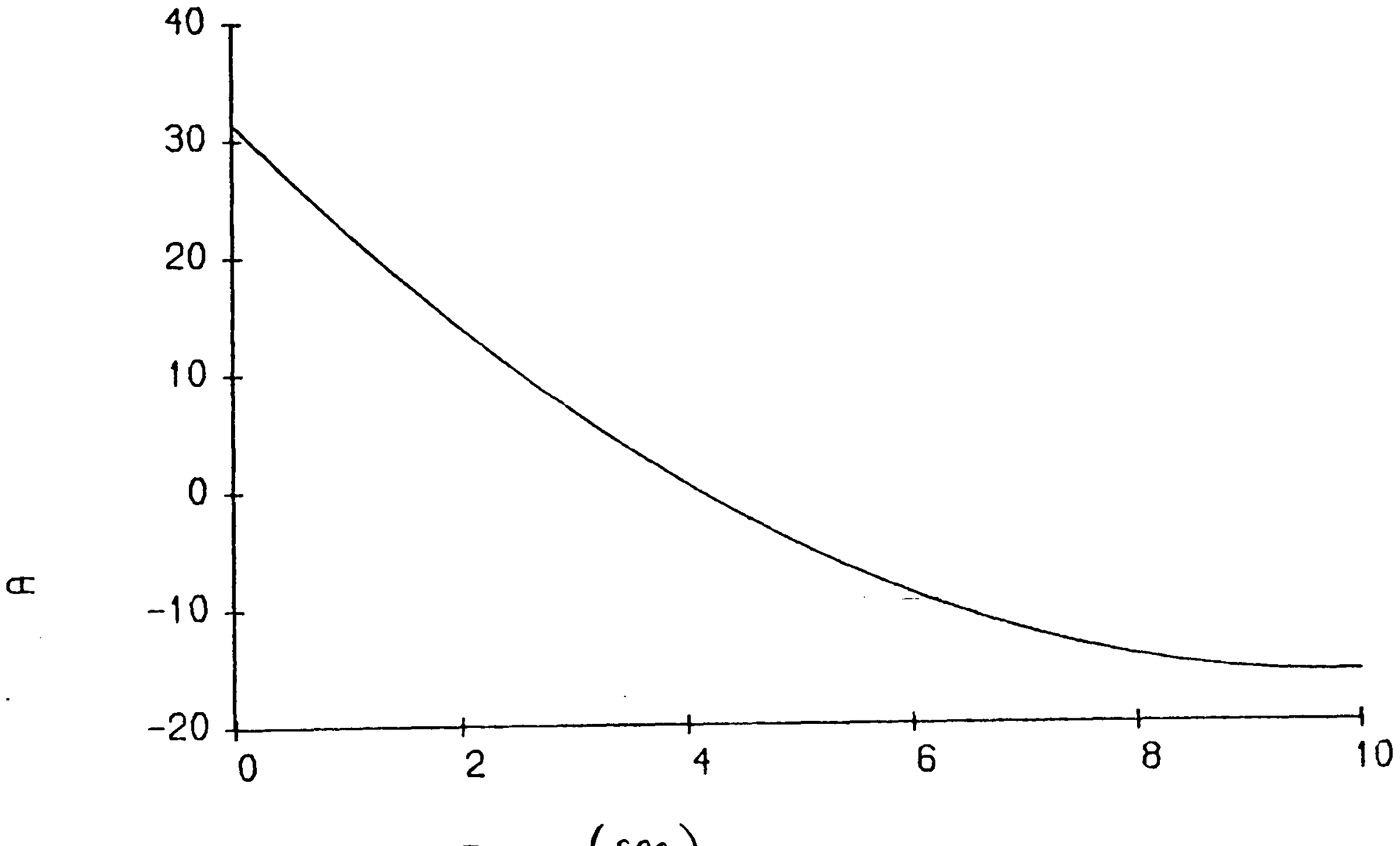


Table 57. Analysis of covariance of the probability of the female beginning to STA during VIB.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	1.70	8.29	0.0010
Linear coefficient	1	1.31	6.39	0.0250
Quadratic coefficient	1	0.12	0.59	NS
Divergence in slopes	138	0.18	0.66	NS
Between positions	69	0.24	1.15	NS
Error	524	0.21		
Linear coefficient =	-0.0972			
Quadratic coefficient =	0.0050			
Y-intercept =	0.3134			

Figure 28. Regression of the probability (x100) of the occurrence of STAs (A) during bouts of VIB (T).



Regression line is curved because computer drawn graph includes effect of non-significant quadratic term.

Table 58. Analysis of covariance of the probability of the female starting to RUN during VIB.

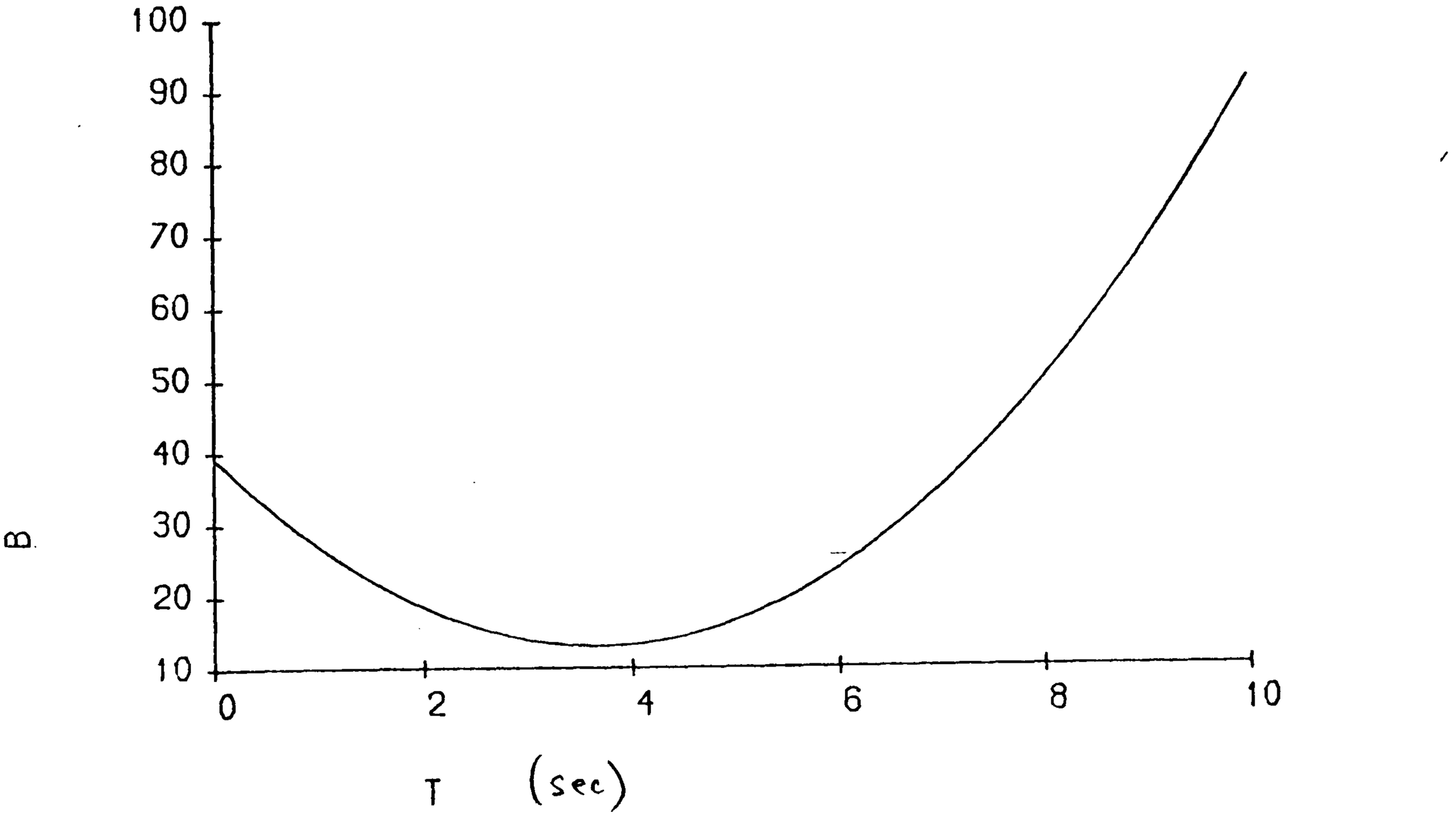
Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	1.47	13.45	0.0010
Linear coefficient	1	1.79	16.39	0.0010
Quadratic coefficient	1	0.57	5.19	0.0250
Divergence in slopes	96	0.15	1.34	NS
Between positions	48	0.05	0.43	NS
Error	426	0.11		

Linear coefficient = -0.1432

Quadratic coefficient = 0.0196

Y-intercept = 0.3919

Figure 29. Regression of the probability (x100) of occurrence of RUN (B) during bouts of VIB (T).



found for the probability of elevating, kicking, extruding or preening during vibration bouts (data not shown).

Non-courtship

There was no significant trend in the probability of starting a bout of standing, elevating or preening while the male was not courting (data not shown). However, there was a significant quadratic decline in the probability that the female would start a bout of running. The decline was the same in all females (Table 59, Fig. 30).

Probability of male behaviour patterns occurring during female ones

Standing

There were no trends in the probabilities of beginning orientation and non-courtship bouts, or in the occurrence of licks and attempted copulations during bouts of standing (data not shown). There was however, a significant quadratic trend in the probability of a bout of vibration starting during standing (Table 60, Fig. 31). All males began with the same y-intercepts but they did not follow the same trend.

Running

The probability that bouts of vibration would begin while the female was running declined quadratically (Table 61, Fig. 32). All males followed the same trend and began with the same y-intercept. The probability of licks occurring during running also declined quadratically with time (Table 62, Fig. 33). Again all males followed the same trend and began with the same y-intercept. There were no trends in the probability of attempted copulations occurring during

Table 59. Analysis of covariance of the probability of the female starting to RUN during bouts of NON.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	1.62	9.89	0.0010
Linear coefficient	1	2.13	13.05	0.0010
Quadratic coefficient	1	1.10	6.71	0.0250
Divergence in slopes	32	0.18	1.12	NS
Between positions	16	0.06	0.39	NS
Error	48	0.16		
Linear coefficient =	-0.1453			
Quadratic coefficient =	0.0092			
Y-intercept =	0.7672			

Figure 30. Regression of the probability (x100) of occurrence of RUN (C) during bouts of NON (T).

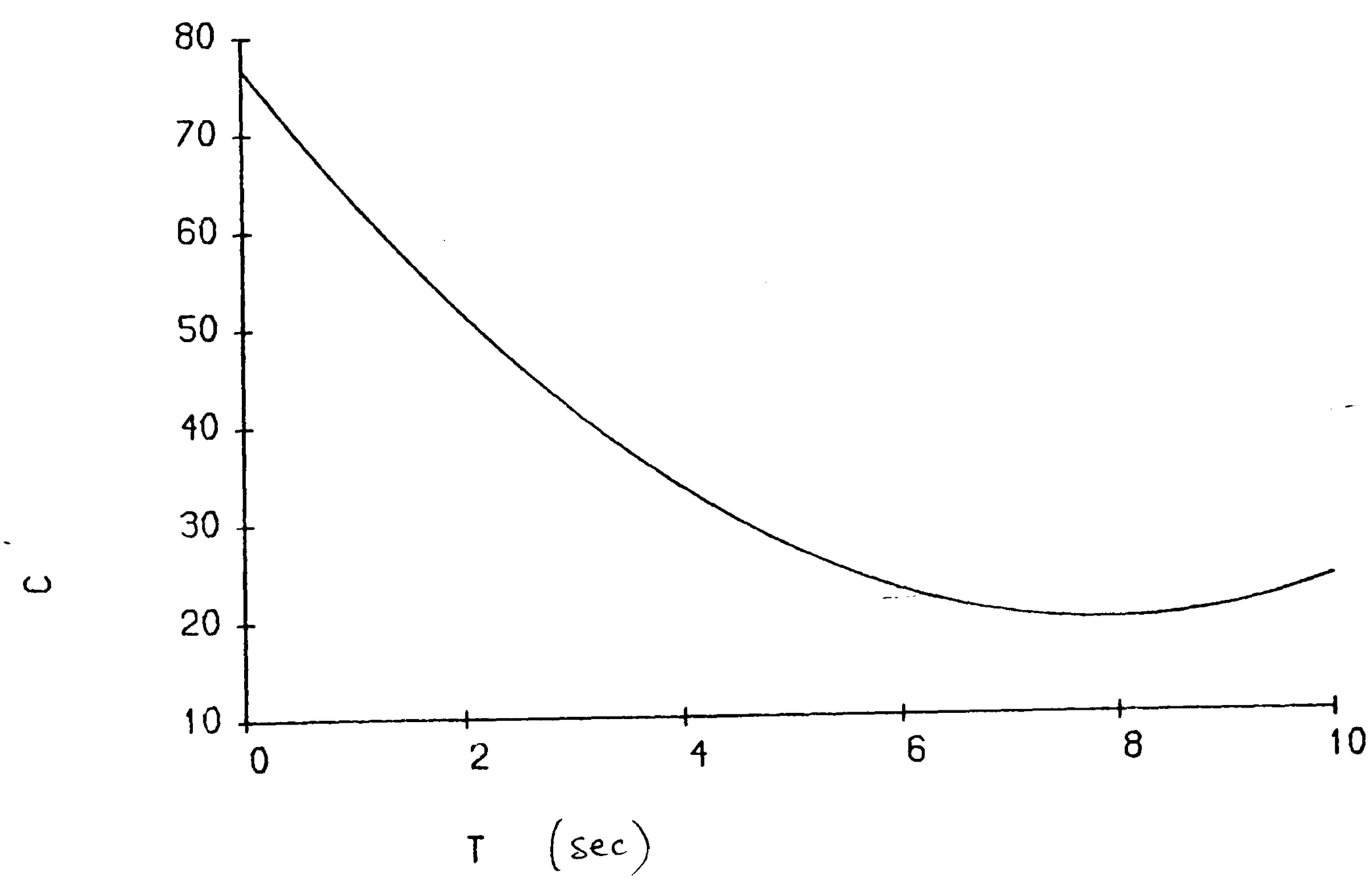


Table 60. Analysis of covariance of the probability of the male starting to VIB during STA.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.66	3.66	0.0500
Linear coefficient	1	0.38	2.13	NS
Quadratic coefficient	1	0.98	5.43	0.0250
Divergence in slopes	72	0.25	1.37	NS
Between positions	36	0.07	0.37	NS
Error	115	0.18		
Linear coefficient =	-0.1107			
Quadratic coefficient =	0.0348			
Y-intercept =	0.3941			

Figure 31. Regression of the probability (x100) of occurrence of VIB (D) during bouts of STA (T).

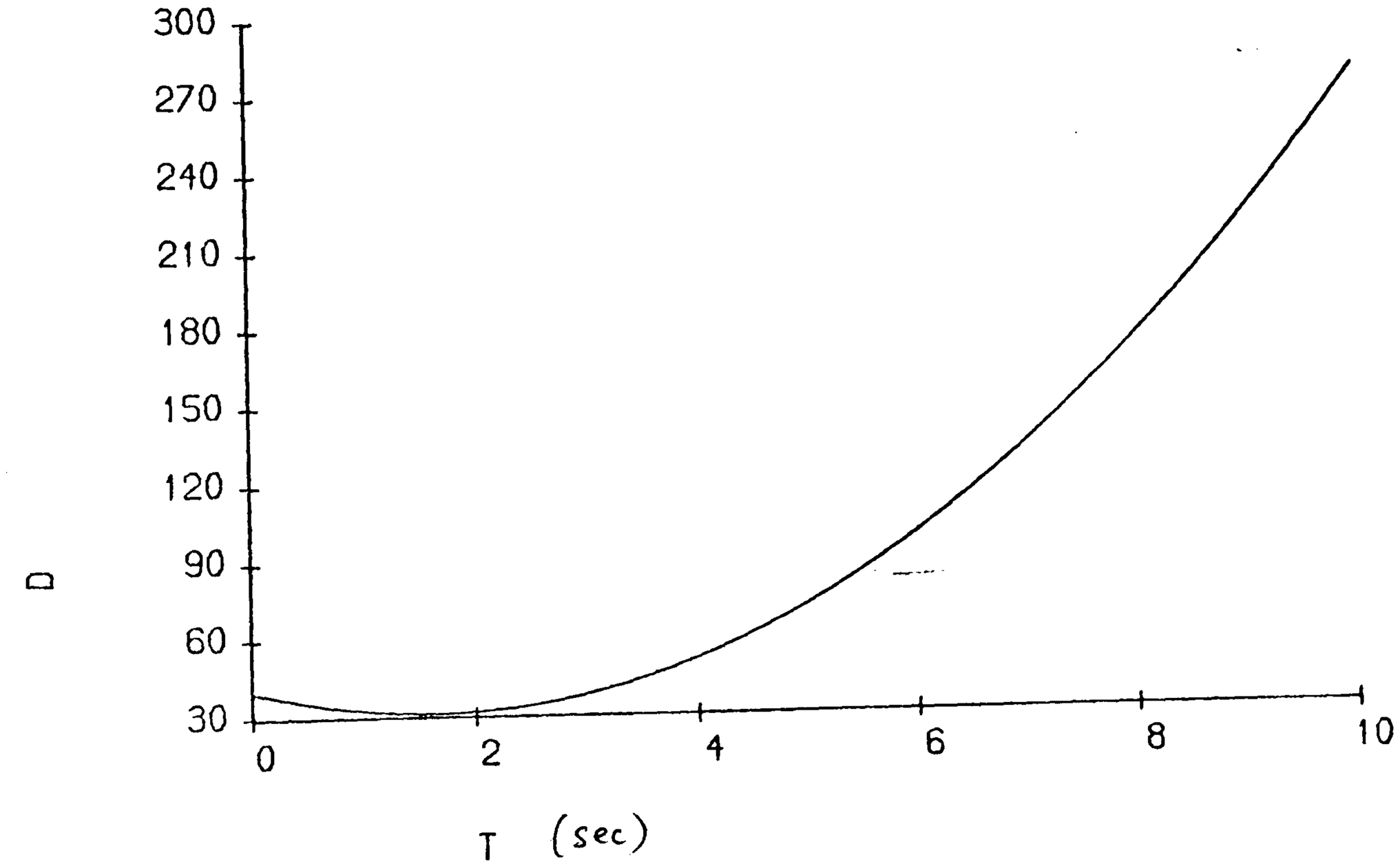


Table 61. Analysis of covariance of the probability of the male starting to VIB during RUN.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	1.36	6.00	0.0500
Linear coefficient	1	2.66	11.76	0.0100
Quadratic coefficient	1	2.22	9.80	0.0500
Divergence in slopes	108	0.23	1.04	NS
Between positions	54	0.28	1.22	NS
Error	250	0.23		
Linear coefficient =	-0.0336			
Quadratic coefficient =	0.0006			
Y-intercept =	0.3162			

Figure 32. Regression of the probability (x100) of occurrence of VIB (A) during bouts of RUN (T).

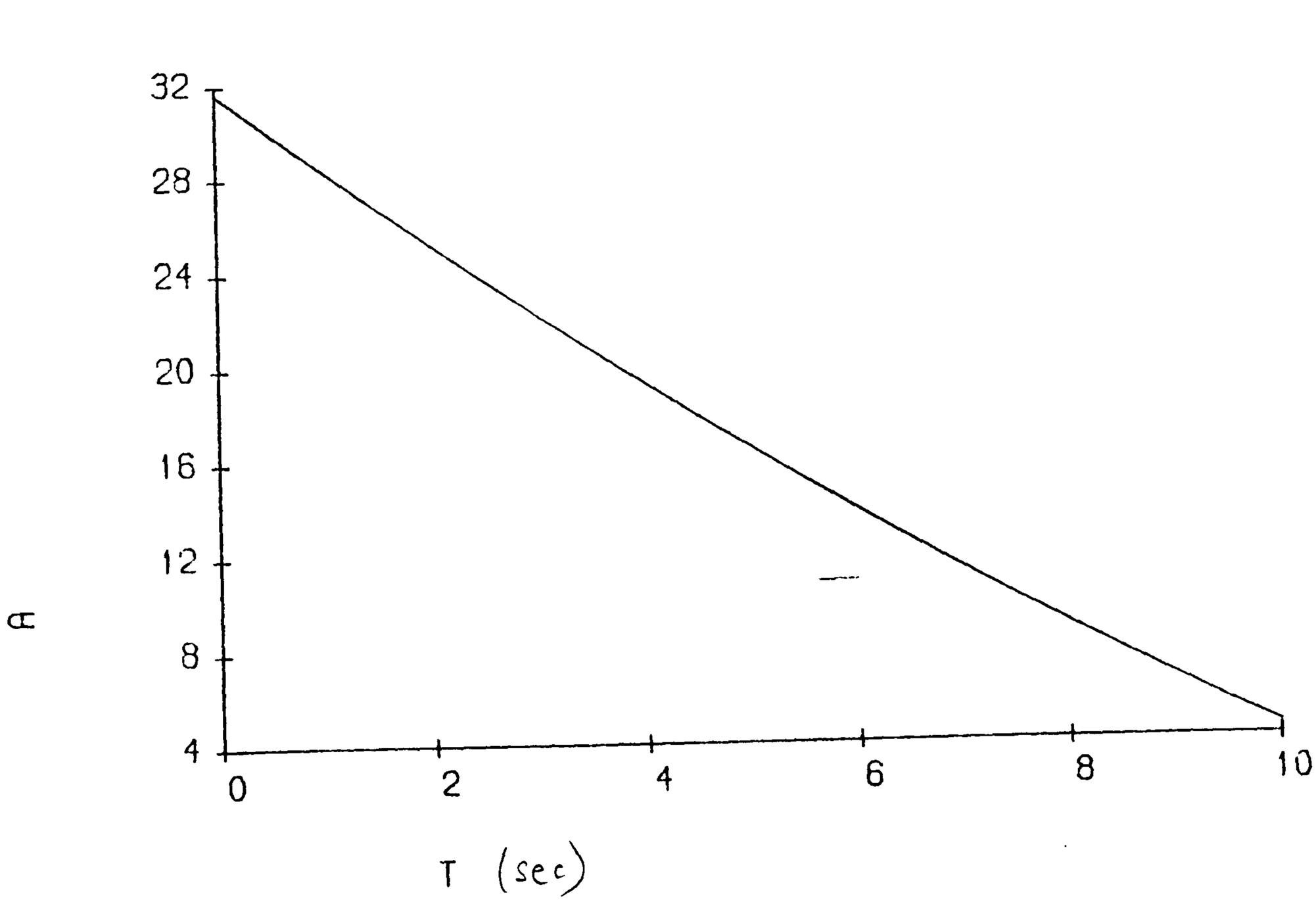
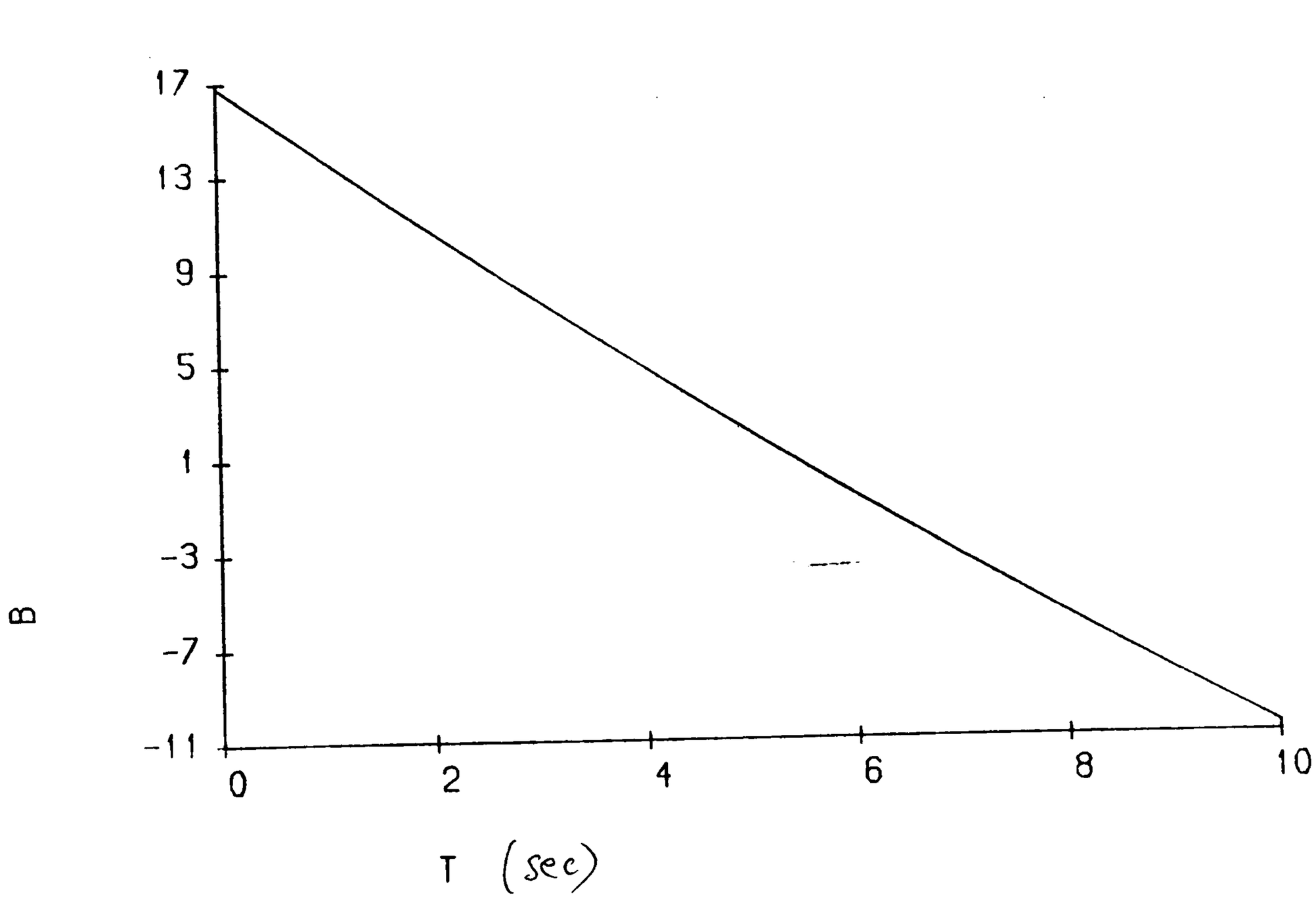


Table 62. Analysis of covariance of the probability of a LIC occurring during bouts of RUN.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.54	3.32	0.0500
Linear coefficient	1	1.04	6.40	0.0250
Quadratic coefficient	1	0.73	4.48	0.0500
Divergence in slopes	44	0.07	0.45	NS
Between positions	22	0.07	0.45	NS
Error	124	0.16		
Linear coefficient =	-0.0320			
Quadratic coefficient =	0.0005			
Y-intercept =	0.1680			

Figure 33. Regression of the probability (x100) of occurrence of LIC (B) during bouts of RUN (T).



bouts of running (data not shown). The probability of a bout of non-courtship beginning during running declined linearly (Table 63, Fig. 34) with all males having the same slope and y-intercept.

Preening

The probability that a bout of orientation would start declined linearly during bouts of preening (Table 64, Fig. 35). All males had the same slope and y-intercept. The probability of a vibration bout beginning during preening however, increased linearly with time, and all males had the same slope and y-intercept (Table 65, Fig. 36).

Discussion

It is somewhat surprising that the vast majority of the significant trends in the probabilities of occurrence of behavioural acts have been declines. This suggests that a change in behaviour in one sex has a general effect on the behaviour of the other sex, and this is confirmed by the results of the analysis of the effect of transitions on the durations of bouts. There also, it appeared that transitions in general, shortened ongoing bouts of behaviour. Hinde (1970) also found that "bout lengths tend to be longer if they are not immediately followed by the next link in the chain - that is, by implication, if they are not interrupted by it".

Crossley (1963) concluded that preening the abdomen, wings and genitalia by the female inhibited the male's courtship. This would appear to have been confirmed by my analysis since the probability of vibrating is low just after the start of a bout of preening. Presumably it rises to its normal level as the inhibitory effect declines with continued preening. Perhaps, the female begins a bout

Table 63. Analysis of covariance of the probability of NON starting during RUN.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.42	2.35	NS
Linear coefficient	1	0.78	4.39	0.0500
Quadratic coefficient	1	0.43	2.41	NS
Divergence in slopes	62	0.11	0.60	NS
Between positions	31	0.15	0.85	NS
Error	171	0.18		
Linear coefficient =	-0.0197			
Quadratic coefficient =	0.0003			
Y-intercept =	0.2051			

Figure 34. Regression of the probability (x100) of occurrence of NON (C) during bouts of RUN (T).

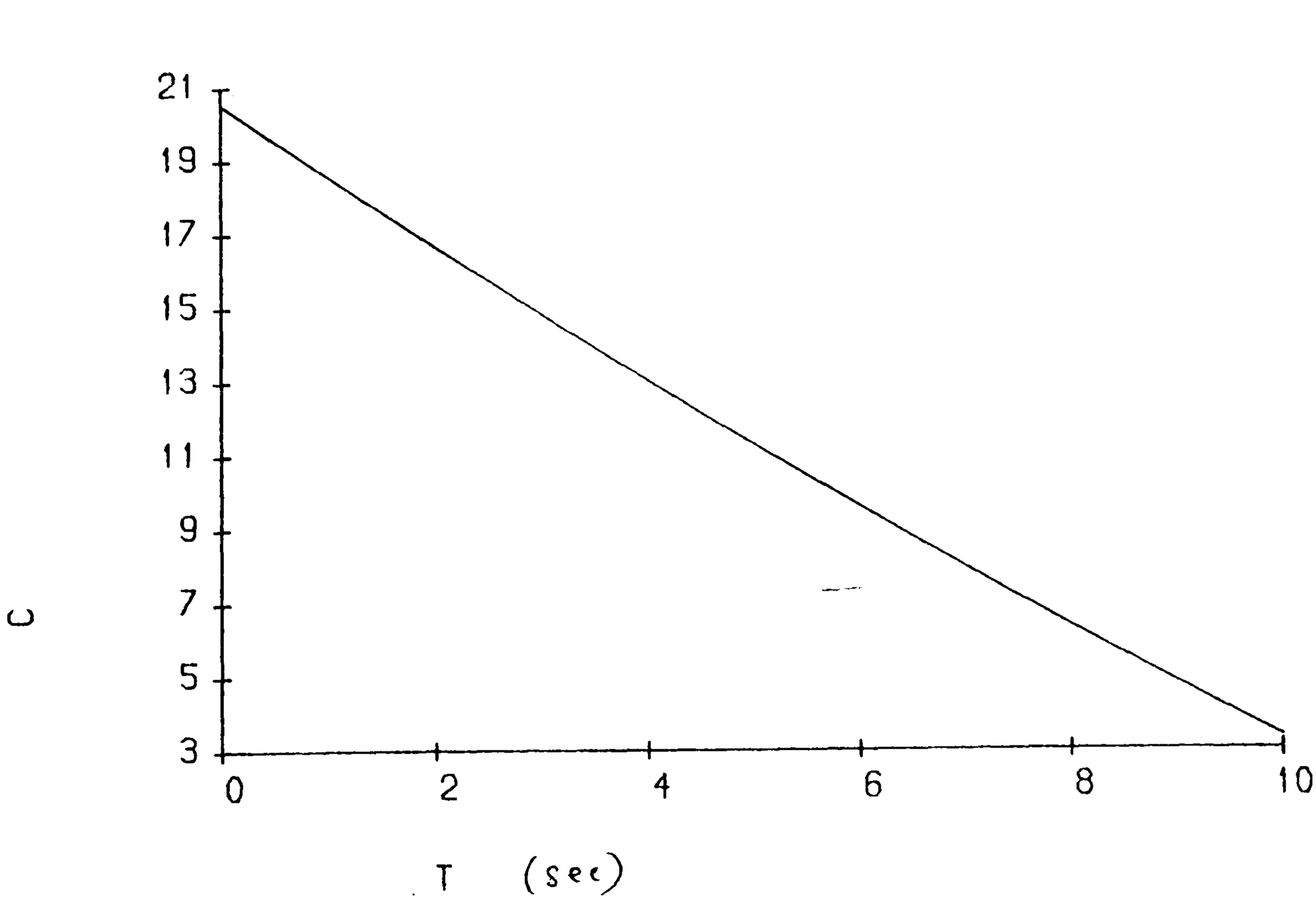


Table 64. Analysis of covariance of the probability of ORI starting during PRE.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.70	3.61	NS
Linear coefficient	1	1.00	5.13	0.0500
Quadratic coefficient	1	0.47	2.41	NS
Divergence in slopes	8	0.16	0.81	NS
Between positions	4	0.14	0.70	NS
Error	15	0.19		
Linear coefficient =	-0.2379			
Quadratic coefficient =	0.0207			
Y-intercept =	0.8992			

Figure 35. Regression of the probability (x100) of occurrence of ORI (D) during bouts of PRE (T).

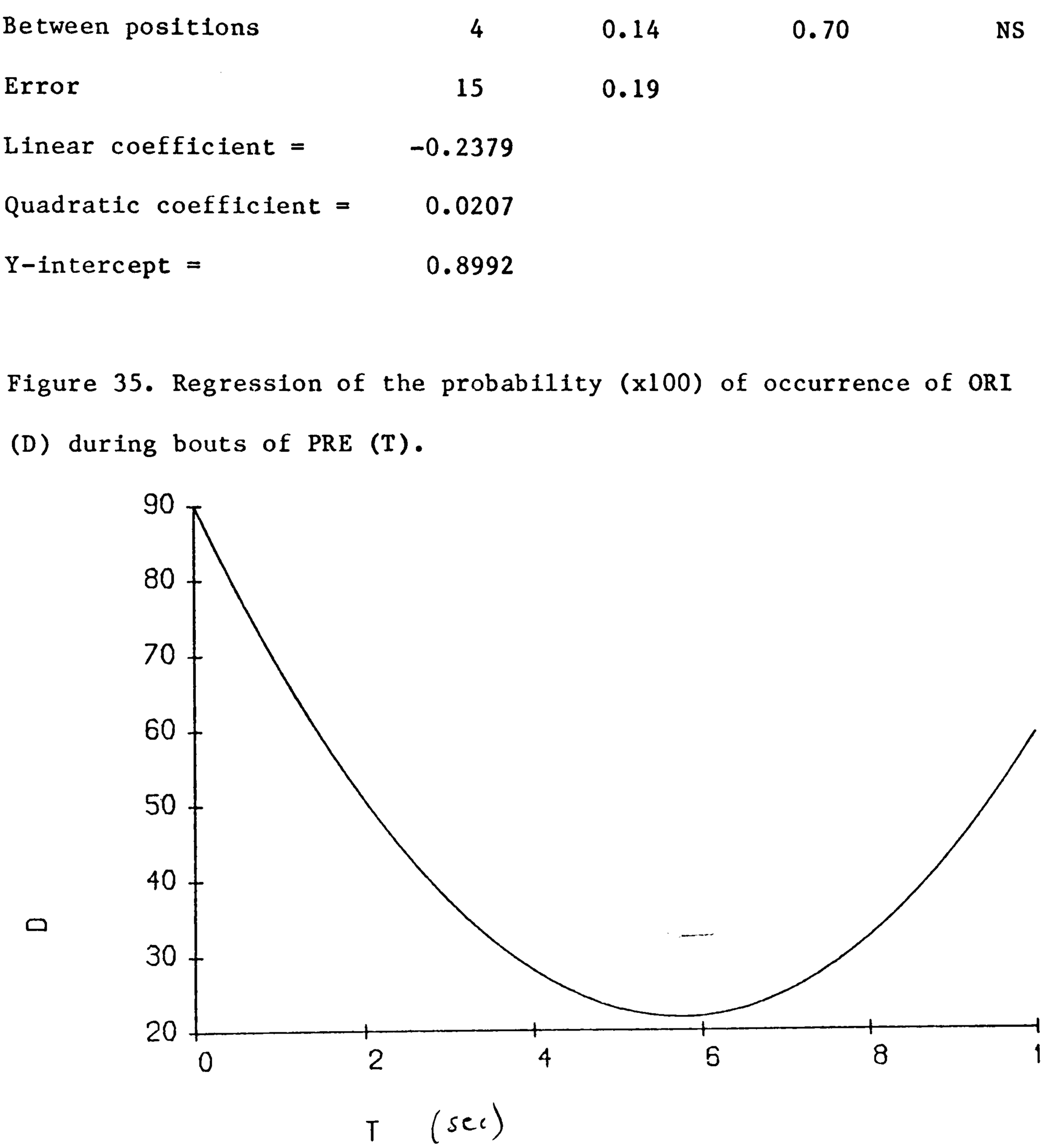
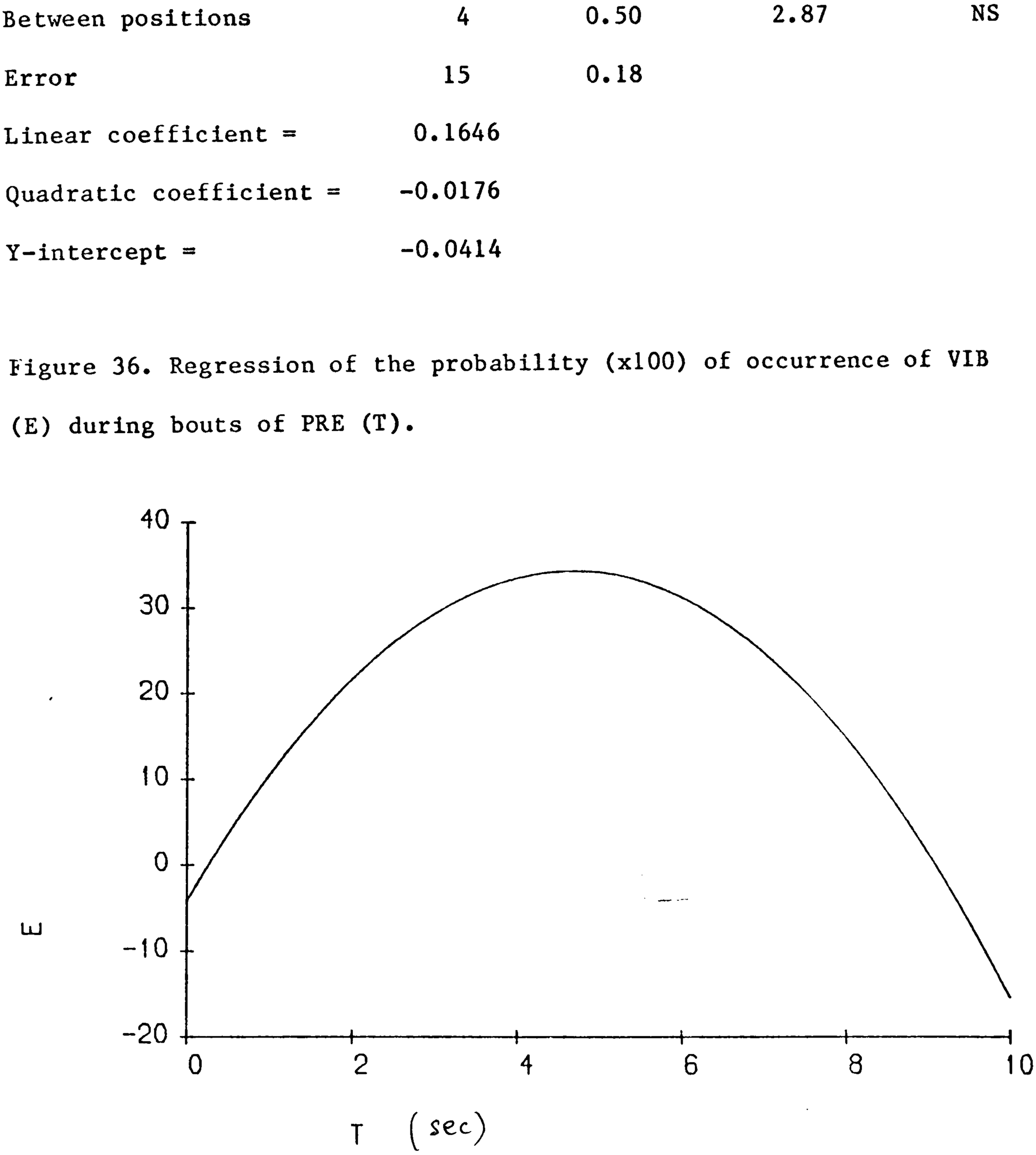


Table 65. Analysis of covariance of the probability of VIB starting during PRE.

Term	D.F.	M.S.	F-Ratio	Prob.
Combined slopes	2	0.65	3.71	0.0500
Linear coefficient	1	0.87	4.94	0.0500
Quadratic coefficient	1	0.38	2.15	NS
Divergence in slopes	8	0.31	1.74	NS
Between positions	4	0.50	2.87	NS
Error	15	0.18		
Linear coefficient =	0.1646			
Quadratic coefficient =	-0.0176			
Y-intercept =	-0.0414			

Figure 36. Regression of the probability (x100) of occurrence of VIB (E) during bouts of PRE (T).



of preening with the abdomen and then proceeds to preen her head. Crossley (1963) suggested that preening the head did not inhibit the male. The inescapable conclusion is that the behaviour of the female does affect that of the male and vice versa.

Chapter 8. Multivariate analyses

The regression analyses of chapter 4 indicated that males differed in both their adjusted mean probabilities of beginning various courtship behaviour patterns and in the way in which their probabilities changed with time. However, a chi-square analysis of the total frequencies per courtship of the various male behavioural acts showed that the males formed a homogeneous set and thus the variability in the frequencies of the courtship behavioural elements could be explained by one underlying variable. This chapter will examine the courtship behaviour of the males with multivariate statistics, particularly principal component analysis (PCA), to discriminate between these conflicting conclusions.

Principal component analysis tries to explain the variance in a set of data by fewer components than the original number of variables: the components are synthetic variables composed of combinations of the original variables. Although the original variables may be significantly intercorrelated, principal components (PCs) are designed so that they are not. To explain 100% of the original variance, the number of PCs required would be equal to the number of original variables. Therefore to reduce the number of components required, first all the original variables are scaled to have zero means and variances equal to one. Then any component that explains less than 1 unit of the variance is not considered to be significant as it does not explain as much of the variance as an original variable.

Principal component analysis of the total frequencies of the male courtship acts indicates that two underlying variables were required to explain the pattern of male courtship behaviour (Table 66). The first component is usually found to be a 'size' indicator

Table 66. Principal component analysis of the total frequencies of male courtship acts.

	Loadings (correlations)					% Variance
	ORI	VIB	LIC	ATT	NON	accounted
PC1	0.94	0.97	0.86	0.80	0.68	73.1
PC2	0.30	0.05	-0.44	-0.53	0.68	20.5

(Blackith and Reyment, 1971) and the male courtship behavioural frequencies were no exception. Component 1 was positively correlated with all five behavioural acts and probably was a measure of the duration of the courtships. The second component contrasted the frequency of licks and attempted copulations with the frequency of orientations and breaks, and was more or less independent of the number of vibration bouts. Thus PCA contradicts the chi-square analysis and reinforces the regression analyses; at least two underlying variables are required to explain the courtship behaviour of male D. melanogaster.

Female behaviour

Principal component analysis of the frequencies of the female behaviour patterns (Table 67) indicates that a minimum of three underlying variables were required to explain female courtship behaviour. For females also the first principal component was a measure of the duration of the courtship. The second PC contrasted the frequencies of elevations, flicks and kicks with the frequencies of standing and extrusions and the third PC contrasted the frequencies of fends with the frequencies of flicks.

Table 68 examines the PCA of both male and female behaviour. Again the first PC was a duration-of-courtship component. Two further components were present in the data. The second PC contrasted the frequencies of licks, elevations and kicks with the frequencies of orientations, breaks and standing. The third component contrasted the frequencies of fends and extrusions with the frequencies of flicks, and was predominantly a female behaviour component. It was not therefore an indicator of sexual interaction. The composition of the second PC suggests that the frequencies of licks and attempted

Table 67. Principal component analysis of the total frequencies of female courtship elements (decimal point omitted).

	Loadings (correlations)							% Variance
	STA	FEN	ELE	FLI	KIC	EXT	PRE	explained
PC1	76	74	81	27	80	30	82	46.6
PC2	37	-09	-33	-29	-36	87	03	17.4
PC3	27	-33	07	87	-22	14	-14	14.8

Table 68. Principal component analysis of male and female behavioural frequencies (decimal point omitted).

	Loadings												% variance
	ORI	VIB	LIC	ATT	NON	STA	FEN	ELE	FLI	KIC	EXT	PRE	explained
PC1	85	91	90	85	58	79	63	82	35	73	31	74	53.2
PC2	40	20	-30	-27	66	33	-15	-38	21	-54	50	-12	14.0
PC3	14	18	16	-09	08	-14	-44	18	71	-05	-55	-26	10.1

copulations in the male were related positively to the frequencies of fends, elevations, kicks and preening in the female.

There is a more efficient method available to determine whether the behaviour of the male is related to that of the female and this is canonical correlation. Table 69 shows the results of canonical correlation analysis between the frequencies of male courtship behaviour patterns and those of the female, and indicates that a) there was a significant correlation between the frequencies of behavioural acts between the two sexes and b) that four independent canonical variates would be required to explain the dependence between the two sets of data (i.e. there were four underlying variables that interrelate the frequencies of male behaviour patterns to the frequencies of female behaviour patterns).

The first canonical variate was a measure of the duration of the courtship as it was positively correlated with the frequencies of all the behaviour patterns (Table 70). No attempt will be made to interpret the remaining canonical variates but it should be noted that this analysis found one more significant variate than did the PCA on the frequencies of both sexes combined.

Principal component analysis of sequences

It has been usual to produce two types of factor analysis on sequential transition data (Wiepkema, 1962; Burton, 1970). One method of analysis has been based on the intercorrelations between the rows of dyad transition tables and the other method has been based on the use of the columns. However, the results of thse two methods do not necessarily confirm each other, and there is some doubt as to whether frequencies or durations or both should be used as the raw data for factor analyses (Balthazart, 1973, 1974).

Table 69. Canonical correlation analysis between male and female courtship elements.

Eigenvalues			Bartlett's test		
Variance accounted	Canonical correlation	Number of eigenvalues	Chi-square	Df	Prob.
		0	362.02	35	0.000
0.874	0.935	1	118.67	24	0.000
0.451	0.671	2	48.28	15	0.000
0.228	0.477	3	17.90	8	0.022
0.136	0.369	4	0.73	3	0.865
0.006	0.079				

Table 70. Loadings of the canonical variates on the original variables (decimal point omitted).

	ORI	VIB	LIC	ATT	NON	STA	FEN	ELE	FLI	KIC	EXT	PRE	% Variance explained
CV1	69	83	99	85	34	66	48	96	38	83	12	61	87.4
CV2	58	38	-01	25	76	61	46	-16	12	-12	69	44	45.1
CV3	38	31	-07	-42	46	30	-40	01	46	43	-21	-30	22.8
CV4	04	21	-01	21	-28	-02	-24	18	-22	-30	52	-13	13.6

The use of separate analyses for the rows and the columns of a transition table assumes that the sequence of behavioural acts has been examined for transitions to acts and for transitions from acts independently. However, the normal method is to analyse the behavioural stream for the frequency of all possible dyads of behavioural acts (i.e. both transitions to and from acts simultaneously).

A sequence transition table exists for each courtship that has been observed. This leads to a third, and possibly more appropriate method for the factor or PC analysis of sequence data. In this method the rows of the matrix would correspond to different courtships and the columns would correspond to different transition dyads. Thus, a dyad transition table with 5 rows and 5 columns would be expanded into 25 columns if all transitions were theoretically possible (or into 20 columns if like-to-like transitions were impossible).

Table 71 details the results of a PCA on such a matrix. This analysis indicates that four PCs were required to explain the sequential organization of male courtship behaviour. Again the first PC was a 'size' component and reflected differences between males in the duration of their courtships. The second PC contrasted transitions between NON and ORI with transitions from VIB to NON and ATT and from LIC and ATT. The third PC cannot be interpreted simply and no attempt will be made to describe it. The last PC correlates most LIC and ATT to NON and may be described as a measure of the frequency of breaks which occur after highly motivated acts (in the scheme of chapter 3).

The most important point to be made from the PCA of sequence transition data is that four PCs were required to explain the organization of male courtship behaviour but only two PCs were required to explain the frequencies of the male behavioural acts

Table 71. Loadings obtained from principal component analysis of male dyad sequences.

Transition	PC 1	PC 2	PC 3	PC 4
ORI -> VIB	0.925	0.197	0.058	0.043
ORI -> NON	0.438	0.696	0.243	-0.334
VIB -> ORI	0.786	0.317	0.193	0.031
VIB -> LIC	0.888	-0.361	-0.154	-0.119
VIB -> ATT	0.386	-0.458	0.684	0.079
VIB -> NON	0.608	0.567	-0.002	0.203
LIC -> ORI	0.663	-0.144	-0.215	-0.160
LIC -> VIB	0.600	-0.399	0.329	-0.245
LIC -> ATT	0.744	-0.284	-0.428	-0.055
LIC -> NON	0.530	-0.102	0.054	0.579
ATT -> ORI	0.773	-0.384	-0.210	-0.225
ATT -> VIB	0.229	-0.407	0.663	0.185
ATT -> NON	0.385	-0.001	-0.440	0.569
NON -> ORI	0.609	0.729	0.189	-0.022
NON -> VIB	0.309	-0.118	-0.440	-0.173
% Variance explained	39.1	16.1	12.3	6.9

during courtship.

Thus far, the unit of analysis has been the entire courtship regardless of the actual duration of the courtships. It is also possible to analyze equal portions of courtship behaviour from each male and female. In this section the courtship behaviour of both sexes has been analyzed for time periods of 15, 25, 35, 45, and 55 second from the start of the observation period. For each of these time periods the following measures have been taken:

Male - total duration of ORI (sec), total duration of VIB (sec), the number of orientation, vibration and non-courtship bouts, and the number of licks and attempted copulations,

Female - total duration of standing, elevation, and extruding (sec), the number of standing, elevating, extruding and preening bouts, the number of fends and kicks.

Males

Table 72 presents the results of PCA of the male behavioural patterns over time. When both behavioural frequencies and durations were included in the PCA, two PCs were required to explain the males' behaviour. The first PC was no longer a measure of duration. This is expected since the time period was a constant. Instead this PC contrasts the frequencies of the courtship behaviour patterns with those of non-courtship. An interesting pattern occurs in the importance of the various measures in the PC. When examined over time the loadings, which are the correlations between the PC and the original variables, for the frequency of ORI, LIC, and ATT rise, and those for the frequency of NON fall. This nicely reflects the trends found previously in chapter 4.

The second component contrasted the frequency of LICs and ATTs

Table 72. Loadings of the principal components derived from male courtship behaviour (constant time periods).

	Time (sec)				
Measure	15	25	35	45	55
First component					
ORI total	0.35	0.61	0.64	0.60	0.59
No. ORI	0.50	0.68	0.76	0.81	0.83
VIB total	0.88	0.87	0.89	0.88	0.89
No. VIB	0.89	0.94	0.93	0.95	0.95
No. LIC	0.68	0.76	0.79	0.82	0.81
No. ATT	0.61	0.64	0.64	0.71	0.72
No. NON	-0.72	-0.50	-0.30	-0.26	-0.18
Second component					
ORI total	0.65	0.46	0.42	0.50	0.53
No. ORI	0.43	0.62	0.57	0.47	0.46
VIB total	0.10	0.08	0.01	0.05	0.02
No. VIB	0.26	0.12	0.08	0.03	0.01
No. LIC	-0.64	-0.39	-0.31	-0.30	-0.35
No. ATT	-0.65	-0.51	-0.49	-0.45	-0.43
No. NON	-0.10	0.53	0.76	0.72	0.71

with the remaining measures. In this PC the loadings of the vibration measures and of the frequency of attempted copulation decreased with time, while that of the frequency of non-courtship bouts increased.

The addition of duration data and the use of a constant time period has resulted in the conclusion that two PCs are required to explain male courtship behaviour. This is the same number as was found when only overall behavioural frequencies were used as the raw data for PCA. Furthermore, the second factors in the two analyses strongly resemble each other. The major difference has arisen in the first PC which has changed from a measure of the courtship duration to a measure of the likelihood that courtship behaviour will occur in any given time period. The other effect of interest is the change in the loadings of the PCs with the original variables. This has reflected the temporal patterning found in the regression analyses of chapter 4.

Females

Table 73 shows the results of PCA on female behaviour using constant time periods. Three PCs were found to explain the variance in female behaviour. The first PC remained a 'size' component. The second and third PCs showed considerable fluctuations with time, but both showed some apparently regular changes, notably in the loadings of fend in PC2 and of standing totals, elevations and extrusions in PC3.

The PCs for the duration and frequency measures in the female did not greatly resemble the PCs for the frequency measures alone. In both cases, however, the analyses indicated that three PCs were required to adequately explain the data.

Table 73. Loadings of the principal components derived from female courtship behaviour (constant time periods).

Measure	Time				
	15	25	35	45	55
First component					
STA total	0.80	0.80	0.76	0.78	0.75
No. STA	0.79	0.82	0.83	0.76	0.76
No. FEN	0.54	0.46	0.40	0.48	0.53
ELE total	0.79	0.64	0.52	0.73	0.76
No. ELE	0.72	0.61	0.53	0.59	0.69
No. KIC	0.51	0.36	0.60	0.35	0.51
EXT total	0.63	0.52	0.73	0.58	0.60
No. EXT	0.64	0.57	0.77	0.64	0.64
No. PRE	0.53	0.44	0.53	0.43	0.38
Second component					
STA total	0.25	0.39	0.36	0.44	0.42
No. STA	0.03	0.18	0.10	0.33	0.08
No. FEN	0.57	0.19	0.61	0.34	0.38
ELE total	-0.26	-0.32	-0.31	-0.40	-0.46
No. ELE	-0.01	-0.32	-0.34	-0.35	-0.53
No. KIC	0.18	-0.01	-0.32	-0.13	-0.28
EXT total	-0.51	-0.39	-0.28	-0.33	0.01
No. EXT	-0.64	-0.43	-0.26	-0.41	0.02
No. PRE	0.62	0.68	0.64	0.54	0.71
Third component					
STA total	-0.19	-0.15	0.03	-0.12	0.02
No. STA	-0.02	0.01	-0.18	0.04	-0.10
No. FEN	-0.29	0.03	0.33	0.21	0.46
ELE total	0.35	0.49	0.66	0.28	0.01
No. ELE	0.61	0.64	0.72	0.62	0.38
No. KIC	0.60	0.53	0.09	0.63	0.60
EXT total	-0.45	-0.69	-0.54	-0.67	-0.73
No. EXT	-0.33	-0.64	-0.51	-0.58	-0.69
No. PRE	-0.39	-0.18	0.00	-0.16	0.31

Combined analysis of male and female behaviour

When the data for both sexes was included in a PCA the results become very difficult to interpret. Table 74 indicates that the number of PCs varied from 5 to 6. Only four of the PCs seem to have been present in all time periods and one of these was a 'size' component. The loadings for the PCs were rather variable and caused considerable difficulty in identifying the same PC in different time periods. Therefore no detailed description will be undertaken of the results of the PCA of the combined data.

Canonical correlations

Canonical correlation analysis of the relationships between male and female behaviour over constant time periods presents a radically different result from that for the entire courtship (Table 75). Only in the 15 sec time period was there a significant correlation between male and female behaviour and then only one canonical variate was significant. There would appear to be little evidence for interaction between the sexes.

Discussion

The results of the chi-square analysis of the frequencies of the male courtship behaviour patterns indicated that only one underlying variable was necessary to explain the differences between males. In this chapter we have seen that multivariate statistical analyses disagree with this conclusion, as did also the regression analyses of chapter 4. Unfortunately the PCAs of male behavioural measures do not agree among themselves on the number of underlying variables present. Thus, frequencies produced 2 PCs, dyad transition sequences produced 4 PCs and frequencies with durations produced 2 PCs. Nevertheless,

Table 74. Number of principal components derived from male and female behaviour (constant time periods).

	Time (sec)				
	15	25	35	45	55
No. of PCs	6	5	5	5	5
% Variance expl.	79.0	71.1	74.5	74.5	75.6

Table 75. Number of significant canonical correlations between male and female courtship behaviour (constant time periods).

	Time (sec)				
	15	25	35	45	55
No. of canonical correl.	1	0	0	0	0

overall it would appear that the evidence favours more than one underlying variable to explain the organization of male courtship behaviour. Discrepancies are also found in the PCAs of female behaviour. Although the number of components required remains constant, the constituents of the principal components is dependent upon whether durations are measured as well as frequencies and upon the duration of the observation period.

In previous sections it has been shown that the behaviour of the two sexes is not independent. Canonical correlation analysis of both frequencies and durations of behavioural acts has shown that the male-female interaction that occurs does not have a large or statistically significant effect. This is basically in agreement with the conclusions of Connolly and Cook (1973). If, as suggested by Bastock and Manning (1955), the behaviour of the female has a cumulative inhibiting effect on the male then this may only be detectable over time periods longer than those examined in the Kansas population of D. melanogaster.

Heiligenberg (1973) and Balthazart (1973) have taken factor analytical methods the furthest in their interpretations and used their results to erect models of the behaviour of their subject animals. In neither instance were changes in time included in their models. This may be particularly important as Balthazart (1974) has found some trends in the behaviour of his fish. Thus, his model of the agonistic behaviour of Tilapia may not be complete and may give an incorrect picture of the behaviour of Tilapia during constant time periods.

The composition of the principal components for male and female behaviour changed with the duration of the time periods over which the analysis was conducted. This reflected to some extent, the

temporal patterning of courtship behaviour found by the regression analyses. However, there are other ways of examining PCs for changes with time. Principal components can be calculated for an initial period, of say 20 sec., and the composition of these components could then be used to estimate the values of the components at other, longer time periods. Trends would then be examined in the values of the PCs rather than in their correlations with the original variables (loadings). This would then show the changes in the underlying variables with time during courtship.

Chapter 9. Summation

The question of stimulatory and inhibitory effects of courtship behaviour on mating success is important to our understanding of the process of sexual selection. Recently, Barrass (1976) has suggested that in Nasonia vitripennis the end result of a courtship does not depend upon the repetition of courtship sequences by the male. Barrass (1976) concluded, "There is no convincing evidence in support of the idea that the courtship of any male insect has a cumulative stimulatory effect on the female."

It has been frequently assumed that male courtship behaviour is summated by the female - i.e. has a cumulative stimulatory effect on the female's sexual receptivity (Richards, 1927; Ewing, 1964; Engelmann, 1970; Manning, 1966). The repetition of various courtship behaviour patterns by the male before copulation has been used as evidence that stimuli are summated e.g., "Initially, her sexual response threshold is high and stimuli from the male's courtship gradually cause it to be lowered to the level where she gives the acceptance posture or simply allows the male to mount and copulate." "Presumably all the various stimuli from the male must have a cumulative effect upon the female, their effects must be stored to some extent and serve to lower her sexual response threshold until it eventually reaches the level where she accepts the male. Probably no one type of stimulus is indispensable and deficiencies in one can be made up by another, i.e. there is 'heterogeneous summation'." (Manning, 1964), "The constantly changing movements of a male's courtship make it certain that what lowers a female's resistance is not a particular stimulus, but the accumulation of a required 'quantity' of stimulation. Presumably this quantity can be composed in different ways but some types of stimulation are more effective

than others ..." (Manning, 1959). However, Kennedy (1966) thought that this evidence was weak and not appropriate for all species.

To determine whether courtship has a cumulative stimulatory effect on a female's receptivity we must make and critically evaluate a number of assumptions. The suggestion that the repetition of male courtship behaviour patterns is evidence for summation rests heavily on the assumption that each repetition of a behavioural act is identical to all other repetitions, in whatever aspect is important to the female. This assumption is not true for D. melanogaster (e.g. bouts of pulse song vary in ipi, and ipi affects the female's receptivity). Furthermore, even if only one behavioural pattern differs quantitatively or qualitatively and all others are constant this may be sufficient to require prolonged courtship before copulation occurs - that is, until the male has produced a variant of the behavioural act that fulfills the female's specifc requirements.

We may contrast the two extreme hypotheses as either pump = summation or trigger = specific requirements (Adler, 1974). It is clear, since males without wings are able to copulate, although it takes them a long time to do so (Ewing, 1964), that song stimuli are not mandatory to achieve copulation. Also since heterospecifc matings between D. melanogaster females and D. simulans males occur although with extreme difficulty none of the courtship stimuli produced by melanogaster males can be considered as absolutely necessary. This assumes that the courtship of the two sibling species is different in all aspects that the female may summate, etc. Forced matings (rape) do not occur with sufficient frequency (Dow and Schilcher, 1974) to explain the mating success of wingless males or of heterospecific matings (Ewing, 1964; Schilcher and Manning, 1975). Thus, females which mate under these conditions do so because they are sexually

receptive. It is known that the amount of courtship stimulation that females require (as measured by courtship time) decreases with age (Cook, 1974) and Dow (1977) found evidence for a decline in female choosiness with age. Both these findings may help explain the occurrence of a low frequency of matings in which the female was sexually receptive under circumstances which would not normally be considered to be sexually stimulating.

Summation hypothesis

One extreme example of a summation hypothesis would assume that all courtship behaviour patterns provide the same sort of stimulation. Each repetition of a particular behaviour pattern (e.g. each LIC) would provide a constant amount of stimulation. The amount of stimulation provided would depend upon the particular behaviour patterns. Stimulation would decay at a particular rate with time and the female would permit mating to occur as soon as her level of stimulation exceeded some threshold value.

Specific hypothesis

A hypothesis which depended upon specific requirements might begin by assuming that each behavioural pattern provided a different type of stimulation to the female and that only repetitions which met the female's specific requirements provided any stimulation at all. Such stimulation might decay with time but could not be increased above the level provided by one correct repetition. The female's specific requirements for each behavioural pattern would have to be met simultaneously before mating would be permitted. As pointed out above this is too extreme for the data. The hypothesis can easily be modified to allow heterogeneous summation (Tinbergen, 1951). Such a

hypothesis would assume that different behaviour patterns provided the same sort of stimulation, that optimum variants of each behaviour pattern provided maximum stimulation and that non-optimum repetitions provided some stimulation, the exact amount being dependent upon their deviation from the optimum. Any number of modifications can be made to the extreme summation and trigger hypotheses to make them more and more alike.

Testing the hypotheses

The most extreme and simplest summation hypothesis can be tested with the data for sine song, pulse song and licks. If the probability of mating remains constant and is independent of the number of repetitions of a particular behaviour pattern then we can conclude that there is no evidence for summation. If summation occurs then the amount of stimulation stored by the female increases with the number of times that a behaviour pattern has been repeated and therefore the probability of mating would increase. Tables 76, 77 and 78 show for sine song bouts and licks in the attached-X strain and for licks in the Kansas strain that the probability of mating is not significantly different from a constant - i.e. the distributions of the number of these behavioural events per courtship are exponential. However, the distribution of the number of pulse song bouts per courtship is significantly different from an exponential (Table 79). In Chapter 5 it was shown that ipi increased to 34 msec during pulse song bouts and that 34 msec is probably the optimum ipi for mating (See also Ewing and Bennet-Clark, 1969; Schilcher, 1976b). Therefore the increase in the probability of mating with repetitions of pulse song bouts cannot be used as evidence for the summation of pulse song by the female (the bouts of pulse song increase in a parameter known to

Table 76. Distribution of the total number of sine song bouts per courtship in the attached X-chromosome strain. Expecteds based on a constant probability of mating (= exponential).

Total no. of sine song bouts	Number of courtships	
	Observed	Expected
0+1	5	7.18
2	4	6.50
3	11	5.88
4	6	5.33
5	5	4.83
6	7	4.37
7	1	3.96
8	0	3.58
9	7	3.25
10	4	2.94
11	1	2.66
12	3	2.41
13+14	1	4.16
15+16	3	3.41
17+18	5	2.80
19 20	2	2.29
21+22	4	1.88
23+24	2	1.54
>24	5	7.03

Chi-square = 26.77, Df = 16, probability of mating after one bout of sine song = 0.094.

Table 77. Distribution of the number of licks per courtship in the attached X-chromosome strain. Expecteds based on a constant probability of mating.

Total no. of licks	Number of courtships	
	Observed	Expected
1	8	15.24
2	11	12.87
3	10	10.87
4	18	9.18
5	11	7.75
6	5	6.55
7	7	5.53
8	5	4.67
9	5	3.94
10	5	3.33
11	1	2.81
12	4	2.37
13	2	2.00
14-18	3	6.21
>18	3	4.68

Chi-square = 20.07, Df = 12, probability of mating after one lick = 0.1556.

Table 78. Distribution of the total number of licks per courtship in the Kansas strain. Expecteds based on a constant probability of mating.

Total no. of licks	Number of courtships	
	Observed	Expected
1	32	35.35
2	33	25.35
3	19	18.18
4	15	13.04
5	7	9.35
6	5	6.71
7	3	4.81
8	3	3.45
9	1	2.47
>9	7	6.29

chi-square = 5.68, DF = 7, probability of mating after one lick = 0.2828.

Table 79. Distribution of the total number of pulse song bouts in courtships of the attached X-chromosome strain. Expecteds based on a constant probability of mating.

Total no. of Pulse song bouts	Number of courtships	
	Observed	Expected
1	1	4.89
2	1	4.58
3	0	4.28
4	1	4.01
5	2	3.75
6	2	3.51
7	2	3.28
8	2	3.07
9	0	2.87
10	3	2.69
11	3	2.52
12	2	2.35
13	7	2.20
14	6	2.06
15	8	1.93
16	2	1.80
17	5	1.69
18	5	1.58
19	8	1.48
20	4	1.38
21	1	1.29
22	0	1.21
23	5	1.13
24	2	1.06
>24	4	15.39

Chi-square = 126.32, Df = 22, probability of mating after one pulse song bout = 0.0644.

affect the probability of mating - ipi).

We can also try to detect summation in the following way. Non-courtship bouts or breaks occur in many courtships. If summation occurs then in courtships containing breaks the last uninterrupted courtship sequence prior to copulation should have fewer licks than those courtships not containing breaks - i.e. there should be some carry-over of stimulation from previous courtship sequences. This was not true for licks or attempted copulations in the Kansas strain (Tables 80,81).

There are two major problems with this method. One is that the breaks may be long enough to cause the loss of all the summation effect. In this case one could regress the duration of the last break with the number of licks required in the last courtship sequence. The expected result when summation is present would be a positive trend. There was no significant trend (Table 82). The second problem is that the quality of a courtship may affect the probability that breaks will occur, such that the courtship of males with breaks is intrinsically less stimulating than that of males without breaks and therefore these males must provide more licks to stimulate the female to mate. In this case the summation effect would be negated

Table 80. Distribution of the number of licks in courtships without breaks and in the last courtship sequence of those with breaks.

	Observed number of licks				
Number of courtships	1	2	3	4	>4
Without breaks	9	8	3	2	2
With breaks	59	15	10	7	10

Chi-square = 5.388, Df = 4

Table 81. Distribution of the number of attempted copulations in courtships without breaks and in the last courtship sequence of those with breaks.

	Observed number of ATTs			
Number of courtships	1	2	3	>3
Without breaks	14	5	3	2
With breaks	71	17	7	6

chi-square = 1.51, Df = 3

Table 82. Regression of number of licks in the last courtship sequence on the duration of the last non-courtship bout.

Measure	Value	Standard error
Regression coefficient	-0.143	0.376
Y-intercept	2.178	0.291

Df = 99, F = 0.14, P>0.70

Chapter 10. Predicting the behaviour of the male

The aim of the mathematical analysis of behaviour is to learn as much as possible about the causation and effects of behaviour patterns so that we may understand their organization and function. One way to test the depth of our knowledge is by attempting to predict an animal's behaviour (Hazlett and Estabrook, 1974a; Hazlett and Bach, 1977). When our predictions are absolutely perfect the collection of data can stop and attempts to simplify our hypotheses may become important (e.g. reduce the number of measurements required). In this chapter I attempt to predict the courtship behaviour of male D. melanogaster.

The stream of courtship behaviour can be resolved into two components - the probability that a behaviour pattern will start at any given time, and once started, its duration. The probability of a behaviour pattern starting can be scored as one (1) at the actual starting times of that pattern and zero (0) at the starting times of all other behaviour patterns. Multiple regression can then be used to choose those variables that are the best predictors of the probability of starting (or the durations). Table 83 lists the variables used to predict probability and durations.

Results

Orientation

Bout duration

The maximum amount of variance explained by the behavioural variables was 23.9% and this used three variables 1) whether the previous behaviour was VIB 2) whether the previous behaviour was LIC 3) whether the previous behaviour was ATT. No other behavioural variables tested (Table 86) were able to increase the amount of

Table 83. Definition of the variables used to predict male courtship behaviour.

Duration of bout

Probability of starting a bout (0,1)

0 - Was previous behaviour ATT (yes = 1, no = 0)

1 - Was previous behaviour LIC (yes = 1, no = 0)

2 - Was previous behaviour ORI (yes = 1, no = 0)

3 - Was previous behaviour VIB (yes = 1, no = 0)

4 - Was previous behaviour NON (yes = 1, no = 0)

5 - Number of previous VIBs

6 - Number of previous ATTs

7 - Number of previous ORIs

8 - Number of previous LICs

9 - Number of previous NONs

10 - Number of previous STAs

11 - Duration of last VIB

12 - Duration of last ORI

13 - Duration of last NON

14 - Mean duration of all previous VIBs

15 - Mean duration of all previous ORIs

16 - Mean duration of all previous NONs

17 - Mean duration of all previous STAs

18 - Total number of previous FLIs+FENs+KICs+EXTs

19 - Is female standing (yes = 0, no = 1)

20 - Time of start of male's behaviour

Table 84. Analysis of covariance of the predictive equation for VIB bouts.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	24	0.25	2.52	0.0001
Zero slope	5	1.64	16.43	0.0000
Error	551	0.10		
Equality of slopes	120	0.08	0.78	0.9504
Error	431	0.11		

Coeff. 1, -0.3655 ± 0.0482

Coeff. 3, -0.0297 ± 0.0105

Coeff. 2, 0.0246 ± 0.0090

Coeff. 6, 0.0283 ± 0.0133

Coeff. 11, -0.0230 ± 0.0101

Proportion of variance accounted by the regression equation can be calculated by summing the various sums of squares (mean squares x degrees of freedom) due to the regression and dividing by the total sum of squares.
This applies to all covariance tables.

variance accounted for. The relationships differed significantly from male to male. For all three variables, the presence of the behaviour acted to increase the duration of the following orientation bout.

However, it will be recalled that orientation bout durations showed cycles in time. Table 22 indicated that four time variables also accounted for approximately 30% of the variance in orientation bout durations. Attempts to include both time variables and behavioural measures together in the same predictive equation failed. This indicates that both sets of variables accounted for substantially the same variance in orientation bout durations.

Probability of orientation

Ten behavioural variables explained 61.2% of the variance in the probability of a bout of orientation beginning (Table 85). Males differed in their predictive equations. Four of the variables are indicators of the previous behaviour and four of the variables are the frequencies of previous behaviour patterns. The probability that the male was orientated was raised if the female was running, the more attempted copulations had occurred, the more breaks had occurred and the more vibration bouts had occurred. It was decreased the longer the duration of the previous vibration bout, the more orientation bouts had occurred and if the preceding behaviour was not non-courtship. Time variables were not significant in increasing the amount of variance explained.

Vibration

Bout duration

Table 84 shows how 34.7% of the variance of vibration bout durations was explained by five behavioural variables. The males did

not differ in their predictive coefficients. If the previous behaviour was a lick the duration of the following vibration bout was decreased. The duration of a vibration bout was also decreased the more vibraton bouts had occurred and the longer the duration of the last vibration bout. It was increased in duration the more orientation bouts had occurred and the more attempted copulations had occurred.

Probability

Eight behavioural variables explained 74.9% of the variance in this measure (Table 87). Males differed significantly in their predictive equations. The probability of a vibration bout starting was increased if the previous behaviour was orientation or lick, the more orientation bouts had occurred, the more attempted copulations had occurred, the more the female had stood and the longer the last vibration bout was in duration. The probability was decreased if the last behaviour was a vibration bout (of necessity since bouts of the same behaviour may not follow each other by definition) and the more vibration bouts had occurred.

Lick

Probability

Seven behavioural variables explained 43.6% of the variance in the probability that a lick would start next (Table 88). The addition of time variables had no significant effect on this and the time variables were not significant in themselves. The males had different predictive equations.

The probability of a lick beginning was increased the more standing bouts had occurred, the longer the previous vibration bout

Table 85. Analysis of covariance of the predictive equation for the probability of bouts of ORI starting.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	20	0.31	2.60	0.0001
Zero slope	10	15.99	135.50	0.0000
Error	1412	0.12		
Equality of slopes	200	0.19	1.77	0.0000
Error	1212	0.11		

Coeff. 2, -0.9141 ± 0.0334

Coeff. 3, -0.3867 ± 0.0323

Coeff. 1, -0.6810 ± 0.0391

Coeff. 0, -0.1207 ± 0.0473

Coeff. 5, 0.0105 ± 0.0083

Coeff. 7, -0.0261 ± 0.0093

Coeff. 9, 0.0461 ± 0.0112

Coeff. 11, -0.0185 ± 0.0074

Coeff. 19, 0.1135 ± 0.0221

Table 86. Analysis of covariance of the predictive equation for ORI bouts.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	13	0.70	3.90	0.0001
Zero slope	3	1.16	6.45	0.0003
Error	413	0.18		
Equality of slopes	39	0.21	1.19	0.2129
Error	374	0.18		

Coeff. 0, 0.3079 ± 0.0744

Coeff. 1, 0.2154 ± 0.898

Coeff. 3, 0.0625 ± 0.0487

Table 87. Analysis of covariance of the predictive equation for the probability of VIB starting.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	20	0.26	3.28	0.0000
Zero slope	8	26.62	332.90	0.0000
Error	1414	0.08		
Equality of slopes	160	0.19	2.80	0.0000
Error	1254	0.07		

Coeff. 2, 0.7444 ± 0.0227

Coeff. 3, -0.0818 ± 0.0225

Coeff. 1, 0.1927 ± 0.0282

Coeff. 5, -0.0331 ± 0.0059

Coeff. 7, 0.0293 ± 0.0051

Coeff. 6, 0.0286 ± 0.0073

Coeff. 10, 0.0136 ± 0.0047

Coeff. 11, 0.0146 ± 0.0060

Table 88. Analysis of covariance of the predictive equation for the probability of occurrence of LIC.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	20	0.17	2.15	0.0023
Zero slope	7	6.74	84.23	0.0000
Error	1415	0.08		
Equality of slopes	140	0.15	2.04	0.0000
Error	1275	0.07		

Coeff. 3, 0.3522 ± 0.0166

Coeff. 1, -0.0499 ± 0.0241

Coeff. 9, -0.0077 ± 0.0043

Coeff. 8, -0.0075 ± 0.0045

Coeff. 10, 0.0120 ± 0.0043

Coeff. 11, 0.0142 ± 0.0060

Coeff. 19, -0.0579 ± 0.0180

and if the previous behaviour was vibration. It was decreased if the previous behaviour was a lick, if the female was running, the more bouts of non-courtship had occurred and the more licks had occurred.

Attempted copulation

Probability

Table 89 indicates that seven behavioural variables accounted for 54.0% of the variance in the probability that an attempted copulation would occur next. Time variables were not significant when included in the predictive equation. The probability of an attempted copulation starting was increased if the previous behaviour was a vibration or a lick and was increased the more licks had occurred. It was decreased the more attempted copulations had occurred, the longer the duration of the last vibration bout, the more orientation bouts had occurred and if the female was running. Males differed significantly in their predictive equations.

Discussion

It must be noted that larger proportions of the variance were accounted by the behavioural measures when predicting probabilities than when predicting bout durations. This may be partly explained by the relatively tight sequential linkage of the male courtship patterns. But it is also possible if vibration bouts had been resolved into sine song and pulse song bouts that the amount of variance explained might have been more. Also the mean duration of orientation bouts was 2.0 sec and of vibration bouts was 1.7 sec. Since human observer reaction times and reliabilities are likely to add as much a + 0.5 sec to these durations (e.g. Dawkins, 1974) the variance of bout durations may have been considerably inflated by

Table 89. Analysis of covariance of the predictive equation for the probability of occurrence of ATT.

Source	D.F.	M.S.	F-Ratio	Prob.
Equality of elevations	20	0.12	2.63	0.0001
Zero slope	7	5.42	123.66	0.0000
Error	1415	0.04		
Equality of slopes	140	0.11	2.90	0.0000
Error	1275	0.04		

Coeff. 3, 0.0281 ± 0.0122

Coeff. 1, 0.4410 ± 0.0181

Coeff. 7, -0.0012 ± 0.0010

Coeff. 8, 0.0238 ± 0.0044

Coeff. 6, -0.0389 ± 0.0072

Coeff. 11, -0.0099 ± 0.0045

Coeff. 19, -0.0666 ± 0.0133

'random' observer effects. There are three specific results of the multiple regression analyses that are of interest. Whether the female was standing or running had an effect on the probability of occurrence of the male behaviour patterns in the expected direction. Licks were more likely if the female was standing. The probability of occurrence of some behaviour patterns was dependent upon the duration of previous bouts of other acts (e.g. licks). Fentress (1972) found similar relationships in mouse grooming. The probability of three of the four behavioural events was related to the number of previous bouts of that event. This suggests that negative feedback processes affect the courtship behaviour of the male.

Although a number of behavioural measures were recorded and used in attempts to predict the male's courtship behaviour there remain a number of measures which might prove useful in future studies:

- pulse song bout durations, frequencies and ipi
- sine song bout durations and frequency
- the male-female distance
- the position of the male with reference to the female (e.g. in front, at side, behind)
- the extent the proboscis is extended on occasions when it does not touch the female (intention lick)
- the extent the abdomen is curled on occasions when it also does not touch the female (intention attempted copulation).

Predicting time to copulation

It is also possible to use the courtship behaviour that has occurred in a given period of time to predict the remaining time till copulation. Both male and female behaviour can be used in this context, and if significant predictions are possible some idea of the

effect of courtship behaviour can be obtained.

Materials and methods

The total durations and the bout frequencies of both male and female behaviour patterns were calculated for the following time periods from the start of the observation periods: 15, 25, 35, 45, and 55 sec. The variables were then extracted by principal components and all the PCs were used to predict the remaining time till copulation by multiple regression.

Results

As shown in Table 90 the proportion of the variance explained by the behavioural variables increased as the time period over which the variables were sampled increased. There was a significant relationship between behaviour and the amount of time remaining before copulation.(Table 91) At best, some 40% of the variance in time to copulation was explained by the behavioural variables. However, it should be noted that calculating means over constant time periods is not the most efficient method. The means of two males may be the same even though their slopes and y-intercepts differ. Also the sample size is continually reduced as the time period is increased. The alternative, of fitting regression lines and using y-intercepts and slopes instead of means also has drawbacks. With this method the shortest courtships would tend to be entirely excluded from the analysis since they would not have sufficient data for the initial regression analysis and long courtships with low frequencies of any behaviour pattern would also be excluded. The sample size would become very small. Therefore, this method was not used.

Table 90. Proportion of variance explained in the remaining time till copulation by the predictive equation.

Sampling Period	% Variance explained	Sample size
15	16.06	121
25	18.42	111
35	16.61	98
45	30.62	74
55	38.63	62

Discussion

The courtship behaviour of the flies is indicative of the amount of time remaining until copulation and therefore can be assumed to play some role in determining that time. This means that the variability in courtship behaviour determines the variability in time to copulation. The exact role of each behaviour pattern cannot be estimated from Table 91 due to the assumptions of multiple regression. If all the variables that determined or affected time to copulation had been known to be included in the regression, then Table 91 would provide the required result. However, since we cannot be certain that pheromones do not play a role and since we know that pulse song and sine song are important, the relationships between the behavioural measures and time to copulation will be biased to an extent determined by the correlation between the measured variables and the unmeasured ones (Snedecor and Cochran, 1967). It is therefore somewhat unsafe to discuss the specific results shown in Table 90.

Table 91. Multiple regression equation for predicting time till copulation. Based on data from the first 55 sec of the observation period.

Variable	Regression coefficient	Mean	Average contribution
ORI total	0.0067	13.54	0.0907
No. ORI	-0.1403	7.45	-1.0455
VIB total	0.0112	9.35	0.1047
No. VIB	0.1056	5.66	0.5978
No. LIC	-0.0889	1.61	-0.1434
No. ATT	0.0335	0.97	0.0324
No. NON	0.1222	2.65	0.3233
STA total	-0.0056	9.59	-0.0541
No. STA	0.0064	4.44	0.0285
No. FEN	0.1270	0.34	0.0430
ELE total	0.1396	0.65	0.0910
No. ELE	-0.1120	1.19	-0.1336
No. KIC	0.0350	0.31	0.0107
EXT total	0.0297	1.14	0.0340
No. EXT	-0.1572	0.48	-0.0761
PRE total	-0.0384	0.83	-0.0320
Y-intercept	1.6422		

Chapter 11. Discussion

The number of ethological studies deeply involved with mathematical and statistical analyses continues to rise at an ever increasing rate (e.g. Dobson and Lemon, 1977; Northup, 1977; Aspey, 1977; van Rhijn, 1977; Machlis, 1977). Perhaps it may be considered to have come of age with the appearance of two books solely devoted to this topic (Colgan, in press; Hazlett, 1977). There are however, misgivings in some quarters (Moynihan, 1973; Beer, 1977) and it is therefore, important to discuss both the positive and negative results of the statistical analyses of the courtship behaviour of D. melanogaster undertaken in this thesis.

Drosophila courtship

Two hypotheses have been based on the courtship behaviour of D. melanogaster: one involved with its organization in the male and the other involved with how it is processed by the female. The regression analyses (Chapter 4) and the principal component analyses (Chapter 7) have clearly shown that the courtship behaviour of D. melanogaster is much more complex than predicted by the model of Bastock and Manning (1955), which therefore, is no longer tenable. At least two underlying factors are required to explain the durations and frequencies of male behavioural elements and four underlying variables are required to explain the sequential organization of the elements.

The test of the summation hypothesis (Chapter 8) is not definitive because the summation hypothesis has never been adequately described and because there are many untested assumptions involved in the test of the one possible hypothesis carried out in Chapter 8. However, the results are suggestive, and do not provide any evidence

whatsoever for the summation hypothesis that was tested. Further tests of storage of stimulation and summation, using experimental song simulation techniques to provide excitatory stimulation unfortunately appear to be producing equivocal results (Morison, personal communication, 1978). The other problem which has received attention in the literature of Drosophila courtship concerns the possible effects that female behaviour might have on male courtship behaviour (Bastock and Manning, 1955; Brown, 1964; Connolly and Cook, 1973; Connolly et al., 1973; Cook, 1974). Bastock and Manning (1955) suggested that, "virgin females do show repelling movements, and that one of these movements certainly has a very strong inhibitory effect when given in its most intense form by males to other males. So although it is not possible to demonstrate any clear correlation between the occurrence of these movements and breaks in the courtship (as we did for extrusion), yet is seems likely that they have some inhibiting effect. It is possible that although they are not strong enough to stop the courtship each time they occur, their inhibitory effect may accumulate until it is, from time to time, able to overwhelm the excitatory factors."

In D. pseudoobscura, Brown (1964) concluded, "The courted female has a large repertory of movements, but these are only performed in response to some action by the male - usually 'contact' behaviour. Except for spreading, all inhibit the male in some way."

More recently it has been found that the male's genotype affects his response to the behaviour of the female (Connolly and Cook, 1973; Connolly et al., 1973) and that male courtship behaviour may be inhibited without any overt display by the female (Cook, 1974). The analyses in Chapter 7 clearly show that in a recently trapped population of D. melanogaster female behaviour can affect both the

duration of male behavioural elements and their sequential organization. The conclusion reached in Chapter 8, was however, that these effects were not large enough to be significant when the male's courtship behaviour was examined over constant time periods. Thus, there is evidence for Bastock and Manning's contention that some female behavioural acts have an inhibitory effect on the male and for Connolly and Cook's conclusion that the inhibitory effects of female behaviour are not significant. It is clear, since the female's behaviour is not highly correlated with that of the male that female behaviour is to a large extent independent of the male's behaviour and is not solely a response to male behaviour.

Analytical

"very often behavioral act frequencies do not fit any standard pattern such as a normal distribution, binomial, etc." (Hazlett and Estabrook, 1974). "At best, a behaviorist can usually only guess the underlying distribution of the events that he is measuring." (Rubenstein and Hazlett, 1974).

These quotations indicate a primary assumption of many ethologists. The assumption of non-normality of data is not usually followed by statistical analysis to determine whether transformation of the data will achieve normality, even though the use of transformations is suggested in nearly all statistical textbooks (Steel and Torrie, 1960; Snedecor and Cochran, 1967; Sokal and Rohlf, 1969; etc.), and even though many ethologists then go on to computer analyse their untransformed data. In fact as can be seen by the by the results of the analyses in Appendix 1 there was no difficulty in normalizing all the behavioural variables measured in this thesis. Should this prove to be of any generality (which is likely as the measures were common

ones), it will allow ethologists with access to a computer to use parametric statistical analyses on their data. This is an advantage since the most efficient and up-to-date techniques are parametric.

Chatfield and Lemon (1970) have emphasized that the statistical analysis of behavioural sequences should only be performed on pooled data when it is known that the population is homogeneous. They suggested that a test of between-individual variation should be made before attempting to analyse pooled data. Slater and Ollason (1972) analysed separately the sequential organization of the behaviour of ten zebra finches, but did not determine whether or not their data were homogeneous. Other workers have simply combined their results from all animals into one transition table (Blurton-Jones, 1968; Fentress, 1972; Baylis, 1976; Lehman and Adams, 1977). There are many factors which may produce heterogeneity in behaviour in populations of animals, among which are food deprivation (Zack, 1974a,b), ovary size (Brothers and Michener, 1974) and body size (Frey and Miller, 1972). The courtship behaviour of both male and female D. melanogaster was heterogeneous (Chapters 4 and 5). Therefore, the assumption of homogeneity would appear to be weak and should be avoided.

Closely related to the possiblity of differences in behaviour among individuals is the possibility of differences in behaviour with time. It has been clear for some time that there are long term changes in the probabilities of the occurrence of behavioural acts. This has resulted in studies of the ontogeny of behavioural elements (e.g. Kruijt, 1964)

Behaviour has been found to change with season (Verberne and Leyhausen, 1976), time of day (Loher, 1974) and age of female (Connolly and Cook, 1973) among others. More recently trends with time have been examined over very short periods. Borchelt (1975)

tested for changes in the probabilities of specific transitions in the dustbathing behaviour of Bobwhite quail and found that there were significant trends; he therefore diagrammed the actual behaviour sequences of a number of birds to show the variability. Balthazart (1974) and Dow et al. (1976) found trends in time in the behaviour of fish fighting in pairs. Short term cycles in the behaviour of zebra finches (<60 min.) were found by Slater (1975) who also had previously found daily cycles in their behaviour (Ollason and Slater, 1973).

As has been pointed out in Chapter 7 the correct interpretation of the results of sequence analysis relies on the presence of stationarity. Stationarity is not present in the courtship behaviour of male or female D. melanogaster. This introduces a bias in the sequence analysis of the total frequencies of behavioural acts (Appendix 2). For male behaviour, the correction for trends in the dyad transition probabilities did not change the conclusions. However, had the correction not been made for the triad transition probabilities, an incorrect conclusion would have been reached; uncorrected triad probabilities were significantly different from random but detrended probabilities were not. To the present, few attempts have been made to take trends into account when analyzing transition probabilities. Oden (1977) has provided one method to do this but his method assumes that individual animals form a homogeneous population and is therefore, not entirely acceptable.

The presence of trends in the data for D. melanogaster also produced biased results in the multivariate analyses. For example, the principal component analysis of male behaviour found two significant components. The composition of these components depended on the unit of time over which the behavioural act frequencies were

obtained. If the unit was the entire courtship, the first component was a measure of the duration of the courtship. If the unit was a constant period of observation, the first component was a measure of the amount of courtship behaviour contrasted with the amount of non-courtship behaviour during that period.

Non-stationarity in behaviour over short time periods may also bias comparisons of different groups, treatments, mutants, etc. and may make the use of means uninformative in such comparisons. Even if constant observations periods are used in such experiments, if trends occur only during periods of courtship, say, and the groups being compared differ in the amount of time they require to start courting, then biased results will be produced if means alone are used for the analysis. At least one example of this has occurred in the Drosophila literature (Burnet, et al., 1971) and the conclusions retracted when regression analysis was undertaken (Burnet, et al., 1977).

Slater (1976) has suggested that non-stationarity in behaviour is a prime requisite for ethologists interested in 'motivational' analysis. This has been well confirmed by Chapter 4 in which regressions in courtship behaviour refuted the unitary motivational model (Bastock and Manning, 1955). It may well be possible that the analysis of trends will provide prove to be a fertile source of information capable of supporting or denying other behavioural hypotheses.

The presence of trends in behavioural variables will also affect attempts to estimate the stereotypy of behaviour patterns (Schleidt, 1976; Bekoff, 1977). It can be easily shown that if two individuals within a species or two species have different slopes for some measure, then the use of means and variances will give a biased estimate of the variability present in the behavioural measure. In

this situation, as in many others throughout the analyses in this thesis, one is faced with the question, what measure (if any) is important to the responding animals - slopes, y-intercepts, both or unadjusted means?

Ethological data are well suited for analysis by multivariate statistical techniques due to the abundance of variables that vary together (e.g. most behavioural measures during courtship) and these methods are being increasingly applied to such data (Huntingford, 1976, etc.). In this thesis I have used principal components analysis which showed that for durations and frequencies of behavioural acts two underlying variables were required. However, once the complexity of sequential organization was considered, four underlying variables were required. It is not unreasonable to find that the number of significant components depends upon the type of data analysed, and it may be that the sequential organization of courtship behaviour is more complex than the organization of durations and frequencies. How many underlying variables would then be required for the analysis of durations, frequencies and transition dyads combined? It is obvious that the frequencies of the individual behavioural elements can be obtained from the frequencies of the dyad transitions. Thus, it may be possible that the principal components for the frequencies of behavioural acts are accounted for in those for the dyad transitions. If this is true, they are certainly no longer capable of simple interpretations.

Multiple regression is a method that is often used by psychologists to predict the values of particular behavioural measures. It has proven successful in D. melanogaster for the prediction of the probabilities of the various male courtship elements, of the durations of the various male courtship acts and of the time to

copulation. It can only be hoped that technical advances in equipment that allow us to measure more behavioural variables with greater accuracy will quickly provide mathematical ethologists with the capabilities for predictions good enough to move on to the next step, of refining and simplifying hypotheses.

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Appendix 1. Transforms

Since the majority of the statistical analyses in this thesis are parametric it is imporatant to establish that the behavioural data meet the assumptions for this type of test. The most important requirement is that the data be normally distributed. Since it is not known whether the behavioural measures are stationary it is not permissible to combine data without regard to time. Therefore, I have chosen one observation (the first) from each courtship which contained at least one sample of that particular behavioural measure.

Table 92 sets out two tests of normality of the behavioural interval measures, i.e. skewness (g1) and kurtosis (g2), for both the raw and logarithmically transformed data. Since the analysis of the data requires 48 statistical tests it is best if a critical probability value lower than the customary 5% is chosen to indicate significance. If the critical value is set at 0.05/48 then the experiment-wise error rate is 0.05 (i.e. one statistical test would be significant by chance in 20 such tables). It is clear that the interval measures are lognormal. It should be noted that the mean and standard deviation of the raw data are approximately equal. This indicates that the intervals are 'random' or are produced by a Poisson process. Schleidt (1974) has indicated that the standard deviation of a logarithmically transformed Poisson process equals 0.5. This is true for the behavioural intervals.

Table 93 shows that a logarithmic transformation also normalizes the durations of the behavioural patterns. Although the mean and standard deviation of the raw data are approximately equal, the standard deviation of the logarithmically transformed data does not equal 0.5. The critical significance level for this table was set at 0.05/32 = 0.0016.

Table 92. Analysis of intervals (sec).

Behaviour	Mean	S. D.	Skewness	Kurtosis	N
ORI	7.95	16.88	6.92*	60.27*	125
VIB	4.85	9.26	5.51*	38.77*	120
NON	7.53	8.64	1.87*	4.07*	77
LIC	14.26	15.96	2.45*	6.75*	93
ATT	19.56	20.86	1.73*	3.19*	79
KIC	12.32	11.99	1.03*	-0.13	22
FLI	14.57	16.12	1.58	2.85	5
ELE	11.15	13.24	2.05*	3.80*	59
EXT	14.10	26.70	2.64*	6.02*	20
PRE	26.51	24.76	1.10*	0.31	28
STA	7.43	9.58	3.00*	13.29*	117
RUN	1.19	0.66	2.32*	7.12*	119

Analysis of intervals (log sec).

ORI	0.52	0.55	0.31	-0.13
VIB	0.33	0.55	0.04	0.11
NON	0.57	0.57	-0.18	-0.94
LIC	0.94	0.46	-0.48*	1.18
ATT	1.04	0.51	-0.36	-0.07
KIC	0.86	0.48	0.02	-1.28
FLI	0.82	0.77	-1.19	1.75
ELE	0.79	0.50	-0.12	-0.22
EXT	0.69	0.58	0.95	0.32
PRE	1.17	0.58	-0.95	0.56
STA	0.56	0.56	-0.12	-0.88
RUN	0.05	0.39	0.81*	0.46

* $P < 0.05$

Normal distributions have non-significant levels of skewness and kurtosis.

Table 93. Analysis of bouts (sec)

Behaviour	Mean	S. D.	Skewness	Kurtosis	N
ORI	1.30	1.31	2.79*	12.59*	125
VIB	1.57	1.43	2.42*	7.17*	125
NON	10.21	18.90	5.85*	44.14*	101
ELE	0.67	0.90	2.67*	7.65*	85
EXT	2.29	3.09	2.79*	9.34*	38
PRE	2.41	2.09	1.13*	0.70	50
STA	1.81	2.66	4.39*	24.00*	124
RUN	13.24	13.74	1.49*	1.81*	125
Analysis of bouts (log sec).					
ORI	-0.05	0.38	0.23	-0.67	
VIB	0.07	0.33	0.32	-0.26	
NON	0.66	0.54	0.25	-0.54	
ELE	-0.41	0.40	1.12*	0.03	
EXT	0.08	0.50	0.21	-0.52	
PRE	0.19	0.46	-0.38	-0.91	
STA	0.04	0.39	0.72*	0.46	
RUN	0.86	0.53	-0.25	-1.01	

* $P < 0.05$

Appendix 2. Serial correlation

Slater and Wood (1977) have indicated that autocorrelation of time sampled behavioural data is biased when the behavioural elements are organized into bouts. I have replicated their sample (p. 738) and can confirm their findings (Table 94). It should be noted, however, that the positive bias in favour of Type I errors is removed when the probability of a transition from type 0 to type 1 behaviour patterns is 0.5 (i.e. whenever only one behaviour is bouted). It should be further noted and emphasised that the lack of independence between consecutive autocorrelations (lag n versus lag n+1) has no effect on the observed number of type 1 errors when the behaviour elements are not bouted. In any series of 100 autocorrelations of lags 1 to 100, 5 correlations are expected to be labelled as significant at the 5% level when the correlations are independent. The number of such pseudo-significant correlations observed with non-independent autocorrelations was not significantly different from the expected number.

Table 94. Simulation of random series of behaviours (0,1). Tested with autocorrelation analysis. Number of behaviours = 240; number of lags introduced = 100.

					Observed		Expected
					mean no.	S.E.	mean no.
					significant	of	significant
P(0->1)	P(0->0)	P(1->1)	P(1->0)	N	(5% level)	mean	(5% level)
0.1	0.9	0.0	1.0	100	3.14	0.19	5
0.05	0.95	0.0	1.0	100	4.94	0.33	5
0.2	0.8	0.8	0.2	10	1.90	0.18	0.5
0.5	0.5	0.5	0.5	10	0.47	0.08	0.5
0.5	0.5	0.5	0.5	100	4.84	0.24	5
0.3	0.7	0.3	0.7	100	5.16	0.26	5
0.9	0.1	0.9	0.1	100	2.73	0.16	5

Appendix 3. Removing trends in sequence analyses

The analysis of sequence contingency tables when there are trends in the probabilities of occurrence of behavioural elements becomes biased. Tables 95, 96, and 97 clearly show that the value of the test statistic, chi-square, is inflated when frequencies are pooled either over time or between individuals, when such data sets are different. Fortunately, the effect appears small for large differences in frequencies. However, it is not too difficult to devise methods to account for such differences.

Differences between individuals

As shown by the tables, if the expecteds are calculated for each table separately and then pooled, the chi-square on the overall table is not inflated. This method has been suggested for homogeneous tables by Everitt (1977). In the case of heterogeneous tables it can be used to test the significance of the population sequence table.

Differences with time

Table 95 and 96 could represent the first and last half of a courtship just as easily as they can represent two individuals. In simple sequential analyses where the data from one courtship was sampled over the entire duration of courtship one is assuming that the probability of occurrence of all the behavioural elements is constant with time. If there are trends then this is not the case. The simplest alternative is to remove the constant probability of occurrence and replace it with a trended probability of occurrence as follows. Let the observed probability of a given behaviour, say Z, occurring at a given position in the sequence of behaviour be either one (1) or zero (0) depending upon whether the behaviour did or did

Table 95. Effect of non-stationarity of behaviour on chi-square tests. Rows are preceding behaviours; columns are following behaviours.

Animal 1

	A	B	
A	9	21	30
B	21	49	70
	30	70	100

chi-square = 0.0

Animal 2 (Table 96.)

	A	B	
A	49	21	70
B	21	9	30
	70	30	100

chi-square = 0.0

Pooled animals (Table 97.)

	A	B	
A	58	42	100
B	42	58	100
	100	100	200

Expecteds from row and column totals of pooled table,

chi-square = 5.12, $P<0.05$.

Expecteds from sum of expecteds of the two sub-tables,

chi-square = 0.0.

not occur at that position. The expected probability of occurrence of behaviour Z at each position can be estimated by fitting a regression to the data e.g. a quadratic regression y = a + bx + cx where y is the expected probability, when there is a trend in the probability of occurrence of Z. This can be done for all the behavioural elements and results in a matrix of probabilities of occurrence for each behaviour pattern at each position. Then the probability of a Z -> U transition as the first transition in the courtship is equal to PeZ1 x PeU2 where PeZ1 is the expected probability of Z at position 1 and PeU2 is the expected probability of U at position 2. The expected number of Z -> U transitions during the entire courtship is the sum of all the probabilities of Z -> U transitions (i.e. N-1, Z -> U transitions). All the expecteds can be obtained in the same manner and since we are summing expecteds the overall chi-square will not be biased.

For higher order sequences one replaces the occurrence of the next lower order transitions with either 0 or 1 and continues the analysis in a similar manner. To be more precise in the regression, one should use constraints such that the expected probabilities are never less than zero, since this is impossible. With such constraints information analysis can be undertaken directly on the probabilities.

Appendix 4. Analyzing single cells in R X C tables

On many occasions the analysis of a sequence table is not complete until significantly divergent cells can be identified. Many approximate methods have been used to identify such cells (Hazlett and Bossert, 1964; Andrews, 1956; Stokes, 1962; Slater, 1974). The statistically correct method is to collapse the entire table into a number of 2 X 2 tables. The number of such tables allowed is equal to the number of degrees of freedom of the overall table (Everitt, 1976). Here I offer my own method.

An N X N table has (N-1)(N-1) degrees of freedom but has N^2 cells. If the expecteds were calculated from theory or from previous knowledge the degrees of freedom would be $N^2 - 1$ and therefore the limiting degrees of freedom per cell would be 1. The critical value for chi-square at, say, 5% increases with increasing degrees of freedom. Therefore, a conservative test of any cell can always be made using the critical value for 1 degree of freedom. However, the chi-square distribution is continuous (Smith, 1950), and is defined for non-integral degrees of freedom. Therefore it is possible to calculate the critical value for $(N^2 - 2N + 1)$ /cell degrees of freedom obtained by dividing the degrees of freedom in the overall table by the number of cells in the table. One can test the accuracy of such an approximation by simulation (e. g. Grizzle, 1967). Professor C. A. B. Smith (University College, London) has been kind enough to provide me with the integral of the chi-square distribution. I have used an approximation (Jahne and Emke, 1944) to calculate the factorials of non-integral degrees of freedom which are required to calculate the critical chi-square. I have then simulated a number of random tables the cells of which have been tested using the critical value. Table 99 shows that the expected number of

Table 99. Simulation of random sequences. Chi-square analysis of single cells with fractional degrees of freedom.

Number of cells	Number of tables	Sample size	No. of significant cells observed	expected
25	20	100	13	25
25	20	125	92	100
25	50	50	274	250
25	100	25	428	500
25	100	25	453	500
25	100	25	434	500
25	100	25	447	500
25	20	100	13	25
25	20	100	28	25
25	50	100	30	62.5
25	50	25	39	62.5
25	50	50	41	62.5
25	50	10	17	62.5
25	50	200	46	62.5
100	50	100	187	250
100	20	500	75	100
100	20	500	78	100

Sample size = number of events per table.

significant cells and the observed number are in excellent agreement. It should be noted that in a 10 X 10 table there are a 100 cells and if one uses the 5% critical level one expects 5 cells to be labelled as significant which are not (i.e. they are random fluctuations). But there might be many more or conversely many less such cells (5 is only the expected number of such cells averaged over an infinite number of such tables). Therefore, it is safer to use a lower critical probability, say 0.0005, such that the probability of 1 cell in the whole table being significant by chance is 0.05 (i.e. 100 X 0.0005 = 0.05).